Organophosphate Insecticides Induced Histopathological and Biochemical Changes on Male Rats

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ABSTRACT

Background: Histopathological changes in some internal organs have been widely used as biomarkers for health evaluation. Histopathological lesions following pesticide chlorpyrifos (CPF) administration in some organs which has prompted us to undertake this study.

Methods: 30 mature male albino rats, weighing 120 – 150 gms, were randomly divided into 3 groups A, B& C. Oral administration of Chlorpyrifos was to groups B and C in dose of 5 and 10 mg/kg B. Wt respectively. Group A kept as control. Body weight of each rat was recorded weekly. Ten animals from each group were sacrificed at end of the experiment to evaluate some biochemical alterations and the histopathological changes in some internal organs (kidney & spleen) and sexual organs (testis and epididymis).

Results: Chlorpyrifos, an organophosphate insecticide, was orally administered to male rats at the doses of 5 and 10 mg/ kg B. Wt for 30 successive days. Rats in Group C which exposed to high dose (10 mg/ kg B.Wt) showed significantly reduced body weight and body weight gain. Weight of different vital organs were significant increases and decrease in the weight of testes (Group B & C) as compared to control. Different tissues of male rats administer CPF (5 mg/ kg B.Wt) showed mild histopathological changes in liver, testis & epididymis while administration of high dose (10 mg/ kg B.Wt) of chlorpyrifos to male rats for 30 days has produced significant pathological changes in liver, testis and epididymis. Chlorpyrifos increased serum transferase enzymes (AST and ALT) activity. Group A shows no pathological changes.

Conclusion: Our study has shown that 30 days administration of chlorpyrifos at dose of 10 mg/kg B.Wt causes severe histopathological changes which leading to damages of some vital organs along with relative decrease in weights of almost all vital organs.

Keyword: Organophosphorus insecticides, chlorpyrifos, histopathology; liver, kidney, spleen, sexual organs & body weight

INTRODUCTION

In recent years, the use of pesticides has common for increasing criticism, with the aspects of public concern including both the possible accumulation of pesticides
residues in food and crops, therefore they could be a source of many biochemical and physiological disturbance in animals and humans (Awasthi and Parkash, 2007 and Shalini et al, 2006).

Goel et al, 2007 mentioned that organophosphorus pesticides are large group of pesticides which are widely used for a variety of agricultural and public health applications and the exposure to these pesticides is known to produce variety of biochemical changes, some of which may be responsible for the adverse biological effects leaves residues on crops and also contaminates reported in man and animals. It could induce adverse effects on the immune system, pancreas, kidney and reproductive systems (Bebe, Panemanogalore, 2003 Lin et al, 2003, and Amer et al, 2000).

Chlorpyrifos is a non-systemic insecticide that effective by direct contact, ingestion and inhalation (Yurumez et al., 2007 and Nolan et al., 1984). Toxicity chlorpyrifos (CPF) in mammalian & animals has increased in recent years. Histopathological lesions have been widely used as biomarkers for health evaluation (Rekha et al., 2013). Few reports regarding histomorphological changes in some vital organs following pesticide chlorpyrifos administration which has prompted us to undertake this study.

Relative organ weight can be the most sensitive indicator for toxicity of experimental compound, as significant differences in organ weight between treated and control animals might be used as a markers to detect early biochemical effects of pesticides before adverse clinical health effects occur. (Michael et al., 2007; Hernandez et al., 2006, Bailey et al., 2004, Wooley, 2003 and Trimbell, 1991). In fact, as a consequence the repeated doses of CPF and CPM, may be lead to significant disturbances in the biochemical parameters and functions of liver and kidney (Mansour and Mossa, 2010 and Verma et al., 2007). The biochemical changes such as total protein, glucose and total lipids contents in living organisms exposed to insecticides may explain their toxicities.

Due to the above facts, our study is designed to evaluate the effect of orally administered Chlorpyrifos at different doses for 30 successive days on kidney and some sexual organs (testis and epididymis) and some enzymatic activity of albino rats.

MATERIALS AND METHODS

Pesticide Details:

Chlorpyrifos are an organophosphorus insecticides which introduced by Giba-Geigy AG (Novartis). It was purchased from the market with Molecular formula C9 H11 Cl3 – NO3 PS

Animals:

Thirty male albino rats weighing 120-150 g. Rats were obtained from the National Institute of Ophthalmology, fed on basal diet and watered ad-libitum.

Experimental design:
Thirty mature male rats (120-150g) were divided into 3 groups. The first group was kept as a control, whereas the second and third groups were administered orally Chlorpyrifo in doses of 5 and 10 mg/kg.b.wt. respectively daily for 30 successive days and the sexual organs weight were recorded.

**Body weight and body weight gain assay:**
The body weight of each treated and untreated rats was recorded every week. The biological value of the treatments was assessed by the determination of its effect on body weight gain (BWG) at the end of each experimental period using the following formula (Rezq and El-Khamisy, 2011):

\[
BWG = Final \ Body \ Weight - Initial \ Body \ Weight
\]

Rats were weighed at the beginning of the study, on Day 0, and then body weight gains were calculated for 0-4 weeks

**Relative organ weight assay:**
At the end of experiment, all rats sacrificed, the sexual organs (testis, epididymis, seminal vesicle and prostate) and some vital organs (liver, spleen and kidneys) from males were carefully dissected out according to (Stanley et al., 2005) as follows:

\[
\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Whole Body weight (g)}} \times 100
\]

**Blood samples:**
It was obtained from each rat at the end of the experimental period, left to clot and the serum was separated for biochemical analysis. The activities of AST, ALT and AP were determined according to the method of Reitman and Frankel (1957) and Roy (1970) respectively.

**Histopathological Examination**
**Sampling**
At the end of the experiment, 10 rats of each group were sacrificed. Rats were decapitated and liver, testis, and epididymis were removed immediately and were fixed in 10% formalin and routinely processed for histopathological evaluation using conventional paraffin embedding technique. Paraffin section of approximately 4-5 µm were stained with hematoxylin and eosin (Boncroft, 2008).

**Statistical analysis:**
The results were subsequently analyzed following the statistical methods established by Snedecor (1982)

**RESULT AND DISCUSSION**

Pesticides have been one of the most effective compounds discovered by man to protect agricultural products from the harm of Pests. Chlorpyrifos is an extensively used organophosphate pesticide and due to its wide-spread use it poses potential
harm to humans (Kavitha., and Venkateswara Rao, 2009). The toxic effects can be attributed to the physiologic features of vital organs. In fact any drug or chemical in the systemic circulation will be delivered to these organs in relatively high amounts. (Griffin et al., 1999 and Nolan et al., 1984).

**Body weight and body weight gains:**

The results tabulated in ([Table 1](#)) indicate a significant decrease in body weight gains of male rats treated with tested insecticide (Group C) compared to control. Our data are in agreement with the results reported by (Mohamed, 2014, Jaiswal and Verma, 2012, Ambali et al., 2011a, Bozkurt et al., 2010, Johnson et al., 2009 and Hancock et al., 2007), who found that significant reduction of body weight gain in rat after administration of chlorpyrifos, and methyl-parathion with no effects on physical or reflex development.

**Relative organ weight:**

The organ weight ratios in toxicological studies is an integral component in the assessment of pharmaceuticals, chemicals, and medical devices (Sellers et al., 2007, Wilson et al., 2001), alterations in body weight may lead to increases or decreases in some organ-to-body weight ratios. The results of ([Tables 2](#)) show that there were significant increases in relative weights of liver, spleen & kidneys in treated male rats (Group B & C) as compared to control; on the other hand, there were significant decreases in weight of the testes of male rats (Group C) compared to control.

Our results are coincided with (Mossa and Abbassy, 2012, Jacobsen et al., 2004, Kang et al., 2004 & Yoshida et al., 1985) who found that administration of chlorpyrifos to male and female rats & mice significantly increase in relative liver and kidney weights in treated animals.

**Biochemical and Histopathological Findings:**

In the present study, rats administered CPF (Group C) showed significant increase of serum activities of AST, ALT and AP ([Table 3](#)). The elevated transferees enzymes denoted the adverse effect of CPF on hepatic function. Our results are confirmed histopathologically as liver of male rats administer of chlorpyrifos (10 mg kg.B.wt) for 30 days showed focal degenerative & necrotic changes which replaced by mononuclear inflammatory cells. Most hepatocytes around necrotic foci have vascular degeneration & some of them have pyknotic nuclei ([Fig 1](#)), vascular degeneration of hepatocytes as well as marked vasculitis of portal blood vessel with swollen of its endothelial cell lining & degenerative changes of its muscular wall ([Fig 2](#)) & in ([Fig 3](#)) liver has marked mononuclear cell infiltration around portal area as well as most hepatocytes having vascular degeneration. Activation of Kupffer cells could be seen. Some hepatocytes showing pyknotic nucleiand portal area with marked mononuclear cell infiltration & bile duct proliferation. The portal blood vessels showing marked vasculitis ([Fig 4](#)). Previous work has reported dilatation of central vein, degradation, congestion, oedema, hyalinosis, fibrosis and necrosis in the liver of rats (Mansour and Mossa, 2010a, 2011). Also, hepatocellular degeneration and necrosis was recorded in rat treated with profenofos The hepatic function tests
confirmed by the histopathological lesions observed in the present study. Degeneration and necrosis in the hepatocytes, inflammatory cells infiltration, and Kupffer cells proliferation were frequently observed in CPF-treated group. These observations indicated marked changes in the overall histoarchitecture of liver in response to CPF. Our results are supported by other studies conducted on CPF and other OP insecticides (Heikal et al., 2011, Mansur et al. 2011, Mansur and Mossa 2010 and Tuzmen et al., 2008).

Repeated exposure of high dose (10mg kg.B.Wt) of chlorpyrifos to male rats for 30 days showed significant patho-morphological changes in sexual organs. The previous results were supported histopathologically as Seminiferous tubules some detachment of spermatogenic cells from its basement membrane & leyding cells have so showing me degenerative changes (Fig 5) as well as Seminiferous tubules showing marked degenerative changes of spermatogenic cells& sever congestion of interstitial blood vessels could be seen accompanied by edema. The interstitial leyding cells have degenerative changes and necrosis (Fig 6). The epididymis of male rats exposed to chlorpyrifos (10 mg kg. B. wt) for 30 days showing some spermatids inside the lumen of its tubules. Edema in the interstitial tissue between epididymal tubules could be seen accompanied by some degenerative changes of interstitial connective tissue(Fig 7) & epididymal tubules have degenerative and necrosis of its epithelial lining as well as detachment of its epithelial ling from basement membrane. Edema between epididymal tubules could be seen(Fig 8). Organophosphorus pesticides have the ability to cross the blood-testis barrier inducing oxidative stress and lipid testes (Okamura et al, 2009, Nahid et al, 2009 Uzunhisarcileli et al, 2007 and Pant and Srivastava, 2003) This in turn may cause degeneration of spermatogenic and leydig cells, which disrupt spermatogenesis. The sperms themselves may also be damaged by oxidative effects of organophosphorus pesticides. Sexual organs of male rats exposed to lower doses (5 mg kg.B.Wt) of chlorpyrifos did not exhibit any significant pathological changes.
Fig 1: Liver of male rats exposed to chlorpyrifos (10 mg kg.B.wt) for 30 days showing focal degenerative & necrotic changes which replaced by mononuclear inflammatory cells. Most hepatocytes around necrotic foci have vascular degeneration & some of them have pyknotic nuclei (H&EX400)

Fig 2: Liver of male rats exposed to chlorpyrifos (10 mg kg.B.wt) for 30 days showing vascular degeneration of hepatocytes as well as marked vasculitis of portal blood vessel with swollen of its endothelial cell lining & degenerative changes of its muscular wall (H&EX400)
Fig3: Liver of male rats exposed to chlorpyrifos (10 mg kg.B.wt) for 30 days has marked mononuclear cell infiltration around portal area as well as most hepatocytes having vascular degeneration. Activation of Kupffer cells could be seen & some hepatocytes showing pyknotic nuclei (H&EX400)

Fig4: Liver of male rats exposed to chlorpyrifos (10 mg kg.B.wt) for 30 days showing portal area with marked mononuclear cell infiltration & bile duct proliferation. The portal blood vessels showing marked vasculitis (H&EX400)
Fig 5 : Seminiferous tubules of male rats exposed to chlorpyrifos (10 mg kg.B.wt) for 30 days showing some detachment of spermatogenic cells from its basement membrane & Leyding cells have some degenerative changes (H&E X 400)

Fig 6 : Seminiferous tubules of male rats exposed to chlorpyrifos (10 mg kg.B.wt) for 30 days showing marked degenerative changes of spermatogenic cells & severe congestion of interstitial blood vessels could be seen accompanied by edema. The interstitial Leyding cells have degenerative changes and necrosis (H&E X 400)
Fig 7: Epididymis of male rats exposed to chlorpyrifos (10 mg kg.B.wt) for 30 days showing some spermatids inside the lumen of its tubules. Edema in the interstitial tissue between epididymal tubules could be seen accompanied by some degenerative changes of interstitial connective tissue (H&EX400)

Fig 8: Epididymis of male rats exposed to chlorpyrifos (10 mg kg.B.wt) for 30 days showing its tubules have degenerative and necrosis of its epithelial lining as well as detachment of its epithelial lining from basement membrane. Edema between epididymal tubules could be seen (H&EX400)
Table (1): Effect of oral administered to chlorpyrifos for 30 successive days on body weight and body weight gain of male rats (n=10)

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose mg/kg.B.Wt</th>
<th>Initial Body weight</th>
<th>Body weight after 30 days</th>
<th>Final weight gain%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-ve</td>
<td>----</td>
<td>172.97 ± 7.356</td>
<td>193.5 ± 2.25</td>
<td>42</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>5</td>
<td>165.7 ± 6.89</td>
<td>188.7 ± 2.11</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>170.03 ± 2.411</td>
<td>199.98 ± 4.58</td>
<td>35*</td>
</tr>
</tbody>
</table>

Value represents mean ± SE of 20 rats, * =p<0.05

Table (2): Effect of oral administration of chlorpyrifos for 30 successive days on sexual organs weight, and some internal organs of male rats.(mean±, n=10).

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose mg/kg.B.Wt</th>
<th>ALT U/L</th>
<th>AST U/L</th>
<th>AP U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>----</td>
<td>37.6±3.5</td>
<td>44.6 ±1.32</td>
<td>79.35 ±1.22</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>5</td>
<td>80.02±4.2</td>
<td>79.0±0.77</td>
<td>79.98±0.45</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>51.45**± 6.98</td>
<td>62.43**±2.99</td>
<td>96.99** ±0.6</td>
</tr>
</tbody>
</table>

significant at**, p<0.01
Table (3): Effect of oral administration of chlorpyrifos for 30 successive days on enzymatic activity in serum of male rats (mean±SE, n=10)

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose mg/kg.B. Wt</th>
<th>Weigh of sexual organs gm/100 gm b.wt</th>
<th>Weight of internal organs gm/100gm.b.wt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Testis</td>
<td>Seminal vesicle</td>
</tr>
<tr>
<td>C-ve</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.73±0.07</td>
<td>0.67±0.05</td>
</tr>
<tr>
<td>chlorpyrifos</td>
<td>5</td>
<td>1.526±0.08</td>
<td>0.5±0.02</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.04**±0.04</td>
<td>0.158***±0.014</td>
</tr>
</tbody>
</table>

Significant at **p<0.01 & *p<0.001
REFERENCE


York, Toronto;
