HEPATOTOXIC VS NEPHROTOXIC POTENTIAL OF CHLORPYRIFOS ON SWISS ALBINO MICE

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ABSTRACT
Pesticides have numerous beneficial effects. These include crop protection, preservation of food and materials and prevention of vector-borne diseases. Chlorpyrifos is still widely used pesticide for crops and farm animals. It induces toxicity through inhibition of acetyl cholinesterase. Thus the present study is designed to evaluate hepatotoxic Vs nephrotoxic potential of Chlorpyrifos on Swiss albino mice. chlorpyrifos was administered at 6 mg/kg b.wt dose for 4 weeks by Gavage method. Sacrifice was done on 1st week, 2nd week and 4th week of chlorpyrifos administration in each group. Urea and Uric acid were increases with increased duration of chlorpyrifos exposure.
Degeneration was observed in hepatic cells and hepatic veins. Glomerulus and bowmens capsule were also degenerated. Thus it is concluded from study that chlorpyrifos causes degenerative changes in both liver and kidney, but it causes more degeneration of glomerulus and bowmens capsule. Degeneration of cytoplasm of PCT and DCT are frequent than cytoplasm of hepatic cells. It is evident from study that chlorpyrifos is more neprotoxic than hepatotoxic.

Key Word: Hepatotoxicity, nephrotoxicity, glomerulus, bowmens capsule.

INTRODUCTION
Pesticides have numerous beneficial effects. These include crop protection, preservation of food and materials and prevention of vector-borne diseases. Pesticides are used extensively throughout the world. Pesticide chemicals can induce oxidative stress by generating free radical and alternating antioxidant levels of the free radical scaving enzyme activity 1.
Chlorpyrifos is still widely used pesticide for crops and farm animals. It induces toxicity through inhibition of acetyl cholinesterase (AChE) but also involves multiple mechanisms besides the inhibition of AChE. Like the other organophosphate, Chlorpyrifos toxicity has been largely associated with irreversible inhibition of acetylcholinesterase (AChE) resulting in accumulation of acetylcholine in the cholinergic receptors. However, other putative mechanisms have been implicated in molecular mechanisms of CPF toxicity. Among these, the induction of oxidative stress has received tremendous attention. Chlorpyrifos intoxication causes a significant decrease in the reduced glutathione (GSH), catalase (CAT) and glutathione-S-transferase (GST) activities. Several antioxidant dietary compound classes have been suggested to have health benefits. Evidence shows consumption of these products leads to a decrease in various pro-inflammatory and/or oxidative stress biomarkers.

CP absorbed through the GI tract, enters the blood stream and reaches the liver, the major site of pesticide metabolism, resulting in liver toxicity. Moreover, CP can also be accumulated in the body tissues, proteins, fats and bones for longer period of time causing additional health hazards.

Thus the present study is designed to evaluate Hepatotoxic Vs nephrotoxic potential of Chlorpyrifos on Swiss albino mice.

2. MATERIALS AND METHODS
2.1 Pesticide
Chlorpyrifos (T_{N}–Dursban) were used at an effective concentration, EC = 20% (w/v).

2.2 Experimental model
Swiss albino mice (Mus musculus) weighing 30±5gm were selected as an experimental model in the present study. The animals were housed at controlled environmental conditions 22±2°C, relative humidity 50±10%, and 12h dark-light cycle. All experimental procedures were conducted as per the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

2.3 Methodology
2.3.1 Chronic Toxicity Studies: Selected pathogen-free mice were sorted and chlorpyrifos was administered at 6 mg/kg b.wt dose level for 4 weeks by Gavage method. Sacrifice was done on 1^{st} week, 2^{nd} week and 4^{th} week of chlorpyrifos administration in each group.
2.3.2 Sub-cellular Studies: Mice were sacrificed from each group for histological analysis. The selected organ is dissected out and washed three times in isotonic saline (0.85 w/v %) and then fixed in 10% neutral formalin solution and the tissue was processed. Slides were stained with Haematoxylin-Eosin (H & E) and examined morphometrical under Light Microscope.

2.4.4 Biochemical Assessment: Blood was collected by orbital puncture and centrifuged to separate the serum to carry out further biochemical analysis. With the separated serum biochemical analysis was performed to establish the effects of chlorpyrifos induced toxicity on urea and uric acid level through standard kit process (Hi Media) by spectrophotometer.

3. RESULT

Urea level in control group of mice was 18.00 ± 1.155 mg/dl. In chlorpyrifos administered group of mice urea level was 33.33 ± 2.404 mg/dl, 44.00 ± 1.155 mg/dl and 52.00 ± 1.528 mg/dl after 1 week, 2 weeks and 4 weeks (Graph: 1).

Uric acid level in control group of mice was 3.333 ± 0.1202 mg/dl. In chlorpyrifos administered group of mice uric acid level was 5.067 ± 0.1202 mg/dl, 6.100 ± 0.1732 mg/dl and 7.267 ± 0.1202 mg/dl after 1 week, 2 weeks and 4 weeks (Graph: 2).

Show liver of control mice with normal hepatic cells. Cytoplasm and nuclear material was normal. Central vein was also normal in structure (Figure: 1). Show liver of chlorpyrifos two week administration mice with frequent vacuolization in hepatic cells. Central veins are degenerated in structure. Haemorrhages was observed in hepatic vein (Figure: 2). Show degenerated hepatic cells with degenerated cytoplasm. Haemorrhages were observed in central vein. Clustered nuclei of hepatic cells were observed (Figure: 3). Show liver of four weeks chlorpyrifos administrated mice with degenerate cytoplasmic material of hepatic cell. Frequent vacuolization were observed. Degenerated nuclei were observed on periphery of central vein (Figure: 4). Binucleated hepatic cells were observed. Heterochromatisation was also observed. Degenerated cytoplasm of hepatic cells was also observed (Figure: 5).

Show kidney of control mice with normal glomerulus and bowmens capsule. PCT and DCT are also normal in structure (Figure: 6). Show section of kidney of two weeks chlorpyrifos administrated mice. Frequent vacuolization were observed in cortex region. Degenerated glomerulus was observed. Dilated Bowmen’s capsules were also observed (Figure: 7).
Degenerated podocytes were observed in Bowmen’s capsule. Cytoplasm of PCT & DCT was also degenerated. Clustered nuclei were observed (Figure: 8). Show kidney of four weeks chlorpyrifos administered mice with dilated Bowmen’s capsule degenerated glomerulus were also observed. Vacuolated space was observed in glomerulus. Dilated PCT & DCT were also observed (Figure: 9). Show kidney of four weeks chlorpyrifos administered mice with dilated Bowmen’s capsule .Degenerated glomerulus were observed with vacuolization. Degenerated cytoplasm was observed in PCT & DCT. Clustered nuclei were also observed on PCT. Degenerated nuclei were observed on DCT (Figure: 10).

**Graph - 1: Urea Level in Serum of mice**

<table>
<thead>
<tr>
<th>Groups</th>
<th>CPF 1 Week</th>
<th>CPF 2 Week</th>
<th>CPF 4 Week</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>mg/dl</td>
<td>40</td>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>

**Graph - 2: Uric Acid Level in Serum of mice**

<table>
<thead>
<tr>
<th>Groups</th>
<th>CPF 1 Week</th>
<th>CPF 2 Week</th>
<th>CPF 4 Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>mg/dl</td>
<td>4</td>
<td>6</td>
<td>8</td>
</tr>
</tbody>
</table>
Figure – 1 Show liver of control mice with normal hepatic cells. Cytoplasm and nuclear material was normal. Central vein was also normal in structure.

Figure – 2 Show liver of chlorpyrifos two week administered mice with frequent vacuolisation in hepatic cells. Central veins are degenerated in structure. Haemorrhage was observed in hepatic vein.

Figure -3 Show degenerated hepatic cells with degenerated cytoplasm. Haemorrhage was observed in central vein. Clustered nuclei were observed.
Figure -4 Show four weeks chlorpyrifos administered liver of mice with degenerated cytoplasmic material of hepatic cell. Frequent vacuolization were observed. Degenerated nuclei were observed on central vein.

Figure -5 Show four weeks chlorpyrifos administered mice with degenerated nuclear material of hepatic cells. Binucleated hepatic cells were also observed. Degenerated cytoplasm was observed.

Figure -6 Show kidney of control mice with normal glomerulus and bowmens capsule. PCT and DCT are also normal in structure.
Figure -7 Show kidney of two weeks chlorpyrifos administered mice. Frequent vacuolization were observed in cortex region. Degenerated glomerulus was observed. Dilated Bowmen`s capsules were also observed.

Figure -8 Show kidney of two weeks chlorpyrifos administered mice with degenerated glomerulus. Degenerated podocytes were observed in Bowmen`s capsule. Cytoplasm of PCT & DCT were also degenerated. Clustered nuclei were observed.

Figure -9 Show kidney of four weeks chlorpyrifos administered mice with dilated Bowmen`s capsule degenerated glomerulus were also observed. Vacuolated space observed in glomerulus. Dilated PCT & DCT were also observed.
Figure -10 Show kidney of four weeks chlorpyrifos administered mice with dilated Boomen’s capsule . Degenerated glomerulus were observed with vacuolization. Degenerated cytoplasm were observed in PCT & DCT. Clustered nuclei were also observed on PCT. Degenerated nuclei were observed on DCT.

4. DISCUSSION
Liver suffered from severe lesions after administration of pesticides. Moreover, haemorrhage was evident inter tubular or sub capsular. This happened as a squeal of liver lesions which leading to lack of clotting factors. Also, observed severe toxicity led to necrosis of renal tubules which were replaced with inflammatory cells. These findings were confirmed with results of Gupta\(^8\), Kherer\(^9\). There is little evidence concerning the effects of organophosphates in the liver of healthy individuals, and the existing researches come to contradictive results\(^10,11\). In present study we also observed degeneration of hepatic cells in chlorpyrifos administered group of mice. Degenerative changes were increased with increased duration of chlorpyrifos exposure.

CPF is thought to be primarily metabolized in the liver by multiple, specific cytochrome P450 enzymes through several reaction pathways CPF elicits a number of additional effects, including hepatic dysfunction, haematological and immunological abnormalities, embryotoxicity, genotoxicity and neurobehavioral changes\(^12,13\). Urea and Uric acid were increases with increased duration of chlorpyrifos in present study.

The toxic effect of profenofos and chlorpyrifos on hepatic lesion leading to congestion and haemorrhages of spleen. Also lymphocytes occurred, which many be affected on the immunity. This findings were confirmed with results of\(^14\). Inhibition of cholinesterase by organophosphoric pesticides or their metabolites plays a key role in toxicity. However, inhibition of other enzymes, such as neuropathy target esterase or other beta esterases and
the direct effects of organophosphates on tissues are also important. Chronic exposure to chlorpyrifos can alter the structural and functional integrity of the kidney, induce oxidative stress, and cause nephrotoxicity, which may lead to renal failure. The glomerular tubules of the kidney were vacuolated due to edema, with excessive toxicity concentration and destruction of the glomerular tubules occurred which may be due degenerative changes. Degeneration of renal tubules resulted from collection of albuminous material lining during its excretion in the urine. In present study degenerated glomerulus and bowmens capsule were observed in chlorpyrifos administered group of mice. Proximal Convoluted Tubule (PCT) and Distal Convoluted Tubules (DCT) were also show degenerated and heterochromatised nucleus. Many vacuolated spaces were observed with degenerated cytoplasm. Necrosis of tubular epithelium, cloudy swelling of epithelial cells of renal tubules, narrowing of the tubular lumen, contraction of the glomerulus and expansion of space inside the Bowman’s capsule were observed in the kidney tissues of fish after exposure.

Thus it is concluded from study that chlorpyrifos causes degenerative changes in both liver and kidney, but it causes more degeneration of glomerulus and bowmens capsule. Degeneration of cytoplasm of PCT and DCT are frequent than cytoplasm of hepatic cells. It is evident from study that chlorpyrifos is more nephrotoxic than hepatotoxic.

5. ACKNOWLEDGEMENT
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6. REFERENCES

5. Ambali SF. Ameliorative effects of vitamins C and E on neurotoxicological, haematological and biochemical changes induced by chronic chlorpyrifos in Wistar rats. PhD Dissertation, 2009; Ahmadu Bello University, Zaria, Nigeria.


