Hepato-renal damage and oxidative stress associated with pirimiphos-methyl exposure in male mice

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Abstract
Objective: For many years, the organophosphorus insecticide pirimiphos-methyl (PM) was used as a valuable tool against several stored-product insect species. However, the knowledge of its toxicity for field applicators, manufacturing workers and public health is very limited in literatures. The present work was therefore undertaken in order to investigate the hepato-renal toxicity associated with PM exposure in mice.

Method: Mice were divided into five groups of six mice each and administered PM at doses of 0.03 (ADI), 10 (NOAEL), 31.05 (1/4 LD₅₀) and 62.11 (1/2 LD₅₀) mg/kg body weight in corn oil for 45 days via oral gavage.

Result: The results revealed that the activities of serum enzymes alkaline phosphatase, alanine and aspartate aminotransferases, and serum levels of total protein, urea and creatinine were significantly elevated (by 13-63% of control activity), whereas the level of serum albumin was significantly reduced (by 15-21%) following administration of 31.05 and 62.11 mg pirimiphos-methyl/kg body weight. In liver and kidney tissues, level of glutathione was significantly reduced by 21% and 63% of control activity, respectively. The intensity of the insults in liver and kidney functions as well as oxidative damage caused by this occupational exposure are represented by biochemical alterations and histopathological findings demonstrated in a dose-dependent manner.

Key Words
Histopathology; Kidney function; Liver function; Oxidative damage; Pirimiphos-methyl

INTRODUCTION
Pesticides are ubiquitous in the environment and have significant economic, environmental and public health benefits by increasing the food production and decreasing the vector-borne diseases [1]. Their initial success was mainly based on their high toxicity, high biological specificity and rapid environmental degradation [2]. Organophosphate (OP) compounds are occasionally used indiscriminately in large amounts causing environmental pollution [3, 4]. Residual amounts of OPs including pirimiphos-methyl have been detected in the soil, water bodies, vegetables, grains and other foods products [2, 5]. However, the health effects caused by this occupational exposure are massive and irreversible in some cases. The widespread use of OPs and the high rates of food contamination could leave humans, animals and birds at high risk of pesticidal toxicity [6, 7].

Organophosphate insecticides represent the most widely group of pesticides that has been shown to have toxic effects to non-target organisms [6-9]. They elicit their extensive toxicities through various mechanisms and pathways. The primary toxicity associated with acute exposure to OP insecticides in humans and animals is the inhibition of acetylcholinesterase (AChE) enzyme [10]. The inhibition of AChE increases the availability of acetylcholine which in turn can stimulate cholinergic receptors producing both nicotinic and muscarinic effects in the organism such as muscle contraction and secretion in many glands [11]. However, they also elicit their toxicities via other mechanisms including cytotoxicity, genotoxicity, immunotoxicity, delayed polyneuropathy, nephrotoxicity, hepatotoxicity, carcinogenicity and reproductive toxicity [11-19]. Furthermore, OP insecticides induced toxic effects that probably occur through the generation of reactive oxygen species (ROS), causing damage to various membranous components of the cell [20].

Pirimiphos-methyl (PM; O-2-[Diethylamino]-6-methylpyrimidin-4-yl] O,O-dimethyl phosphorothioate) is one of the most commonly used grain protectants globally. It is an OP compound, with a wide margin of safety to mammals and a broad spectrum of insecticidal activity [21]. Arthur et al [22] declared that PM has been on the market for many years and used as a valuable tool against several stored-product insect species. It is rapidly absorbed, metabolized and
excreted in rats. The major metabolite of PM is 2-ethyl amino-4-hydroxy-6-methyl pyrimidine [23]. Many previous investigators such as Ngoula et al [24] reported that PM caused reproductive toxicity in male rats manifested by a decrease in body and reproductive organs weight after 90 days of treatment. Also, Mantle et al [25] reported that exposure of male rat to PM resulted in a negative effect on the proteolytic enzyme activities in rat heart, kidney, brain and liver tissues.

Pirimiphos-methyl is a cheap pesticide widely used in the world and particularly in Africa to protect food against pests. It is rapidly spread in the environment. Therefore, there is a possibility that it could affect the health of humans and wildlife in their natural habitats. To the best of our knowledge, little is known on the effects of PM on human health, the present work was therefore undertaken in order to investigate the dose-dependent hepato-renal toxicity associated with PM exposure using the criteria of body and relative organ weight changes, biochemical and histopathological parameters.

MATERIALS AND METHODS

Chemicals

Pirimiphos-methyl (95% purity) was obtained from TaeGeuk Corporation (Cheongju, Republic of Korea). All other chemicals were of reagent grades and were obtained from the local scientific distributors in Egypt.

Animals

Male mice weighing 24.5 ± 1 g, obtained from the Animal Breeding House, National Research Center (NRC), Dokki, Cairo, Egypt, were kept in well-ventilated plastic cages, provided with pellets, water ad libitum and subjected to a 12:12 light:dark photoperiod. All animal procedures were approved by the NRC Local Ethical Review Committee and were carried out in accordance with the “Guide for the Care and Use of Laboratory Animals”.

Experimental design

Mice were divided into five groups of six mice each: Group 1 (control) received corn oil as a vehicle; group 2 (ADI, acceptable daily intake) received 0.03 mg PM/kg body weight (BW)/day; group 3 (NOAEL, no observed adverse effect level) received 10 mg PM/kg BW/day; group 4 (1/10 LD₅₀) received 31.05 mg PM/kg BW/day; and group 5 (1/20 LD₅₀) received 62.11 mg PM/kg BW/day through oral intubations. All the doses used in the study were selected from a pilot study and represent ADI, NOAEL, 1/10 and 1/20 LD₅₀ doses according to the published values of Tomlin [26]. Dosages of PM in corn oil were freshly prepared and administered at a fixed volume of 0.1 ml/mice/day for 45 days adjusted weekly for BW changes.

Data collection

At the end of experiment, blood samples were drawn from the retro-orbital venous plexus of the animals into plain tubes. Within 20 min of blood collection, the sera were drawn after centrifugation at 3500 rpm for 10 min at +4°C. The sera were kept in a deep freezer (-20°C) for biochemical analysis. Portions of liver and kidney from all animals in each group were homogenized in 50 mM Tris-HCl buffer (pH 7.4) containing 1.15% potassium chloride. The homogenate was centrifuged at 10,000g for 15 min at 4°C. The collected supernatants were used for the estimation of the activities of superoxide dismutase (SOD) and catalase (CAT), the contents of reduced glutathione (GSH) and the amount of lipid peroxidation (LPO).

Body and relative organ weights

The body weight has been recorded on the initial day of experiment and also on the day of sacrifice (45th day) for the different groups. The increment in body weight was presented as percentage and estimated according to Equation 1. After blood collection, the mice were sacrificed by cervical dislocation. The liver and kidney were quickly removed, cleaned and weighted individually. Then, the organ/body weights ratios were presented and estimated according to Equation 2.

Equation 1:

\[
\text{Percent of BW gain} = \left( \frac{\text{Final BW} - \text{Initial BW}}{\text{Initial BW}} \right) \times 100
\]

Equation 2:

\[
\text{Relative organ weight} = \frac{\text{Organ weight}}{\text{Final BW} \times 100}
\]

Biochemical measurements

All biochemical measurements were determined using commercial kits in accordance with manufacturers’ instructions using a spectrophotometer (UV-2401PC; Shimadzu, Kyoto, Japan). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea, creatinine, total protein, albumin, SOD, CAT, LPO and GSH kits were purchased from the local distributor Biodiagnostic Company (Giza, Egypt).

Histological study

Small pieces of liver and kidney tissues in each group were collected in 10% neutral buffered formalin. These tissues were embedded in paraffin wax. Sections of 5 µm thickness were cut and stained with hematoxylin and eosin (H&E) for light microscopy at x400 magnification. Two slides were prepared for each mice organ; each slide had two sections. Ten field areas for each section were selected and examined for histopathological changes. The organ fields were scored as follows: normal appearance (-); minimal cellular disruption in less than 1% of field area (+); mild cellular disruption of 1-30% of field area (++).
moderate cellular disruption of 31-60% of field area (+++); severe cell disruption of 61-90% of field area (+++); and very severe cellular disruption of 91-100% of field area (++++) [27].

Statistical analysis
The results were expressed as means ± SEM. All data were done with the Statistical Package for Social Sciences (SPSS) version 11.0. The results were analyzed using one way analysis of variance (ANOVA) followed by Duncan’s test for comparison between different treatment groups. Statistical significance was set at P < 0.05.

RESULTS

Signs of toxicity
During the treatment period, no death was recorded in any of the experimental groups. General weakness, huddling and hair loss were observed in $\frac{1}{40}$ and $\frac{1}{20}$ LD₅₀ PM-treated groups. These observed signs were related to the cholinergic syndrome in organophosphate poisoning.

Body and relative organ weights
Administration of ADI, NOAEL, $\frac{1}{40}$ and $\frac{1}{20}$ LD₅₀ doses of PM for 45 days resulted in a significant decline (P < 0.05) in body weight gain as compared to the control group (Fig.1A). Simultaneously, a significant increment (P < 0.05) was observed in the relative liver (Fig.1B) and kidney (Fig.1C) weights compared with the control group, in a dose-dependent manner. The most increase in relative liver and kidney weights was recorded by the highest dose of PM ($\frac{1}{20}$ LD₅₀) which accounted to 7.01% and 2.28% for relative liver and kidney weights, respectively, compared with the corresponding control values for relative liver (4.67%) and relative kidney (1.66%) weights, respectively (Fig.1B&C).

Biochemical measurements
Serum ALT, AST and ALP activities were used as biochemical markers of early hepatic dysfunction, while urea and creatinine were used as biochemical markers of early kidney dysfunction. However, tissues SOD, CAT, LPO GSH were used as biochemical markers for oxidative damage.

The present results revealed that PM at doses of $\frac{1}{40}$ and $\frac{1}{20}$ LD₅₀ induced statistically significant (P < 0.05) increases in the activities of ALT, AST and ALP enzymes (Table 1) compared with the control value. The most significant increase in the activity of ALT, AST and ALP enzymes was achieved by the highest dose of the pesticide used ($\frac{1}{20}$ LD₅₀). The percent of these increases were estimated and accounted to 46.3, 24.7 and 42.7% for ALT, AST and ALP, respectively, as compared with the corresponding control values.

Similarly, the administration of the two high doses of PM induced significant increases in the serum concentrations of urea and creatinine (Fig.2A&B), while induced a significant decrease in serum total protein and albumin concentration compared to the untreated group (Table 2). Except on urea, ADI treatment has insignificant effects on the aforementioned biochemical parameters; however, NOAEL treatment has statistically significant effects only on ALT, ALP and urea compared to the control group.

Figure 1. Percent of body weight gain (A) and percent of relative liver (B) and kidney weights (C) of mice exposed to different doses of pirimiphos-methyl for 45 days. Bars represent the group means of 6 mice ± SD. Bar values not sharing superscripts letters (a, b, c, d) differ significantly at P < 0.05.

Figure 2. Serum urea (A) and creatinine (B) concentrations of mice exposed to different doses of pirimiphos-methyl for 45 days. Bars represent the group means of 6 mice ± SD. Bar values not sharing superscripts letters (a, b, c, d) differ significantly at P < 0.05.
Table 1. The effects on the activity of ALT, AST and ALP enzymes in serum of mice exposed to different doses of PM

<table>
<thead>
<tr>
<th>Treatments</th>
<th>PM doses (mg/kg)</th>
<th>ALT (U/ml)</th>
<th>AST (U/ml)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>45.89 ± 1.4a</td>
<td>39.75 ± 0.67a</td>
<td>74.97 ± 5.57a</td>
</tr>
<tr>
<td>ADI</td>
<td>00.03 mg/kg</td>
<td>46.73 ± 4.23b</td>
<td>40.35 ± 2.15b</td>
<td>77.91 ± 4.94b</td>
</tr>
<tr>
<td>NOAEL</td>
<td>10 mg/kg</td>
<td>51.89 ± 2.22ab</td>
<td>40.55 ± 2.01a</td>
<td>93.22 ± 4.64b</td>
</tr>
<tr>
<td>1/4 LD₅₀</td>
<td>31.05 mg/kg</td>
<td>56.13 ± 3.61b</td>
<td>45.22 ± 1.25b</td>
<td>101.92 ± 6.09c</td>
</tr>
<tr>
<td>1/2 LD₅₀</td>
<td>62.11 mg/kg</td>
<td>67.15 ± 4.08b</td>
<td>51.07 ± 3.08b</td>
<td>107.01 ± 6.61b</td>
</tr>
</tbody>
</table>

Each value is a mean of 6 mice ± SD; values not sharing superscripts letters (a, b, c) differ significantly at *P < 0.05; ADI: acceptable daily intake; NOAEL: no observed adverse effect level; PM: pirimiphos-methyl; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase.

Table 2. The effects on the level of serum total protein, albumin and globulin of mice exposed to different doses of PM.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>PM doses (mg/kg)</th>
<th>Total protein (mg/dl)</th>
<th>Albumin (A) (mg/dl)</th>
<th>Globulin (G) (mg/dl)</th>
<th>A/G ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>6.91 ± 0.39a</td>
<td>3.22 ± 0.16a</td>
<td>3.67 ± 0.25a</td>
<td>0.88</td>
</tr>
<tr>
<td>ADI</td>
<td>00.03 mg/kg</td>
<td>6.62 ± 0.66ab</td>
<td>3.12 ± 0.21ab</td>
<td>3.5 ± 0.64ab</td>
<td>0.92</td>
</tr>
<tr>
<td>NOAEL</td>
<td>10 mg/kg</td>
<td>6.67 ± 0.46ab</td>
<td>3.04 ± 0.15ab</td>
<td>3.64 ± 0.33ab</td>
<td>0.84</td>
</tr>
<tr>
<td>1/4 LD₅₀</td>
<td>31.05 mg/kg</td>
<td>7.83 ± 0.22c</td>
<td>2.72 ± 0.12c</td>
<td>5.11 ± 0.3bc</td>
<td>0.53</td>
</tr>
<tr>
<td>1/2 LD₅₀</td>
<td>62.11 mg/kg</td>
<td>8.28 ± 0.14c</td>
<td>2.54 ± 0.2c</td>
<td>5.74 ± 0.21b</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Each value is a mean of 6 mice ± SD; values not sharing superscripts letters (a, b, c) differ significantly at *P < 0.05; ADI: acceptable daily intake; NOAEL: no observed adverse effect level; PM: pirimiphos-methyl.

Table 3. The effects on the activity of antioxidant enzymes and the level of lipid peroxidation and glutathione in liver tissue of mice exposed to different doses of PM for 45 days.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>PM doses (mg/kg)</th>
<th>SOD (U/g tissue)</th>
<th>CAT (U/g tissue)</th>
<th>LPO (nmol/g tissue)</th>
<th>GSH (mmol/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>436.3 ± 9.08a</td>
<td>329.7 ± 6.45a</td>
<td>221.6 ± 5.45a</td>
<td>165.5 ± 3.63a</td>
</tr>
<tr>
<td>ADI</td>
<td>00.03 mg/kg</td>
<td>433.4 ± 6.57a</td>
<td>285.3 ± 8.27b</td>
<td>252.8 ± 6.62b</td>
<td>161.8 ± 5.39a</td>
</tr>
<tr>
<td>NOAEL</td>
<td>10 mg/kg</td>
<td>420.5 ± 6.17a</td>
<td>247 ± 9.03c</td>
<td>283.8 ± 5.79b</td>
<td>129.2 ± 3.87b</td>
</tr>
<tr>
<td>1/4 LD₅₀</td>
<td>31.05 mg/kg</td>
<td>382.8 ± 8.8b</td>
<td>233.2 ± 7.98c</td>
<td>279.1 ± 9.13c</td>
<td>114.5 ± 3.3e</td>
</tr>
<tr>
<td>1/2 LD₅₀</td>
<td>62.11 mg/kg</td>
<td>242.7 ± 11.73c</td>
<td>182 ± 7.01d</td>
<td>336.2 ± 8.54d</td>
<td>74.9 ± 3.17a</td>
</tr>
</tbody>
</table>

Each value is a mean of 6 mice ± SD; values not sharing superscripts letters (a, b, c, d) differ significantly at *P < 0.05; ADI: acceptable daily intake; NOAEL: no observed adverse effect level; PM: pirimiphos-methyl; SOD: superoxide dismutase; CAT: catalase; LPO: lipid peroxidation; GSH: reduced glutathione.

Table 4. The effects on the activity of antioxidant enzymes and the level of lipid peroxidation and glutathione in kidney tissue of mice exposed to different doses of PM for 45 days.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>PM doses (mg/kg)</th>
<th>SOD (U/g tissue)</th>
<th>CAT (U/g tissue)</th>
<th>LPO (nmol/g tissue)</th>
<th>GSH (mmol/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>529.3 ± 11.56a</td>
<td>308.4 ± 5.19a</td>
<td>197.2 ± 4.85a</td>
<td>157.5 ± 3.31a</td>
</tr>
<tr>
<td>ADI</td>
<td>00.03 mg/kg</td>
<td>523.8 ± 11.37a</td>
<td>280.5 ± 7.32b</td>
<td>221.8 ± 5.11b</td>
<td>147.1 ± 4.9a</td>
</tr>
<tr>
<td>NOAEL</td>
<td>10 mg/kg</td>
<td>498.3 ± 12.62a</td>
<td>267.5 ± 7.81b</td>
<td>252.6 ± 5.15e</td>
<td>117.5 ± 3.52b</td>
</tr>
<tr>
<td>1/4 LD₅₀</td>
<td>31.05 mg/kg</td>
<td>449.8 ± 12.77b</td>
<td>239.7 ± 5.44e</td>
<td>262.3 ± 7.05e</td>
<td>104.1 ± 3.54e</td>
</tr>
<tr>
<td>1/2 LD₅₀</td>
<td>62.11 mg/kg</td>
<td>363.3 ± 11.73f</td>
<td>202.4 ± 5.78d</td>
<td>306.2 ± 7.34d</td>
<td>78.2 ± 3.26d</td>
</tr>
</tbody>
</table>

Each value is a mean of 6 mice ± SD; values not sharing superscripts letters (a, b, c, d) differ significantly at *P < 0.05; ADI: acceptable daily intake; NOAEL: no observed adverse effect level; PM: pirimiphos-methyl; SOD: superoxide dismutase; CAT: catalase; LPO: lipid peroxidation; GSH: reduced glutathione.

A significant decline (P < 0.05) was found in activities of SOD and CAT and in the content of GSH in liver and kidney tissues of mice treated with PM (1/4 LD₅₀ and 1/2 LD₅₀) than the control group. Simultaneously, a significant increment (P < 0.05) was recorded in LPO level in liver and kidney tissues of mice treated with all the tested doses of PM for 45 days of exposure (Tables 3&4).

Histopathology

Light microscopic examination of H&E stained slides of vehicle control animals showed normal histological structure of hepatic lobule and renal parenchyma. Livers of animals treated with different doses of PM on gross examination were seen with Kupfer cells activation, vacuolization of hepatocytes, focal hepatic necrosis and portal infiltration with inflammatory cells (Fig.3A-H). Simultaneously, Kidney sections were seen with cystic peritubular inflammatory cells infiltration, cystic dilatation of renal tubules, interstitial nephritis, vacuolization of epithelial lining tubules and atrophy of glomerular tuft and perivascular edema (Fig.4A-H). Moreover, microscopic examinations showed the severity of either hepatic changes or renal changes (Table 5).
Figure 3. Photomicrographs of the liver sections of mice. Control group (A): normal histological structure of hepatic lobule; ADI group (B): cystic dilatation of bile duct and portal infiltration with inflammatory cells; NOAEL group (C): Kupffer cells activation and focal hepatic necrosis associated with mononuclear infiltration; 1/40 LD$_{50}$ group (DE): Kupffer cells activation, vacuolization of hepatocytes and focal hepatic necrosis; 1/20 LD$_{50}$ group: sinusoidal leukocytosis and extramedullary megakaryocytosis (F). Kupffer cells activation and karyomegaly of hepatic nuclei (G) and portal infiltration with inflammatory cells (H). Magnification: x400; Stain: H&E.
Figure 4. Photomicrographs of the kidney sections of mice. Control group (A): normal histological structure of renal parenchyma; ADI group (B): cystic peritubular inflammatory cells infiltration; NOAEL group (C): peritubular mononuclear inflammatory cells infiltration and cystic dilatation of renal tubules; 1/10 LD₅₀ group: dilatation and congestion of renal blood vessels (D) and congestion of glomerular tuft (E); 1/50 LD₅₀ group showing interstitial nephritis (F), vacuolization of epithelial lining tubules (G) and atrophy of glomerular tuft and perivascular edema (H). Magnification: x400; Stain: H&E.
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Table 5. The severity of the reaction in liver and kidney tissues of mice treated with different doses of pirimiphos-methyl according to the histopathological alterations.

<table>
<thead>
<tr>
<th>Histopathological alterations</th>
<th>Control</th>
<th>ADI</th>
<th>NOAEL</th>
<th>%LD50</th>
<th>%LD20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kupffer cells activation</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Focal necrosis</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Vacuolation of hepatocytes</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Karyomegaly</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Portal infiltration with inflammatory cells</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Congestion of renal blood vessels</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Peritubular inflammatory cells infiltration</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Cystic dilatation of renal tubule</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Focal tubular necrosis</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

AD: acceptable daily intake; NOAEL: no observed adverse effect level. +++ severe; ++ moderate; + mild; nil.

DISCUSSION

The tremendous increase in the production and extensive use of OP insecticides has a profound impact on the environment and creates unexpected hazards to any organism including man. They have been shown to interfere with membrane dependent processes, oxidant and antioxidant status and enzyme activities of experimental animals [28-31].

In terms of toxicology, it is well documented that the alterations in body and organ weights are sensitive indicators of the detection of potentially toxic chemicals [9, 29-31]. In our study, oral administration of different doses of PM resulted in a significant decline in body weight gain and an elevation in relative liver and kidney weights of mice. The reduction in body weight gain may be attributed to (i) reduced food consumption (untabulated data) and/or (ii) the overall increased catabolism of lipids and proteins consequence to the direct effect of OPs [9, 15]. These results corroborate with data from many previous researchers who reported similar results with different OP insecticides in rats [5, 17, 29-31] and in mice [32, 33]. However, the significant increase in liver and kidney mass as percent of body weight may be suggested to be due to the hepato-renal parenchyma edematous reaction to injury. The potentially reactive derivatives of oxygen, ascribed as ROS such as superoxide, hydrogen peroxide and the hydroxyl radical are continuously generated inside the human body as a consequences of exposure to a lot of exogenous chemicals in our ambient environment and/or a number of endogenous metabolic processes involving redox enzymes. Under normal circumstances, the ROS generated are detoxified by the antioxidants present in the body and there is equilibrium between the ROS generated and the antioxidants present [34]. Harmful effects caused by ROS occur as a consequence of an imbalance between the formation and inactivation of these species. However, owing to ROS overproduction and/or inadequate antioxidant defense, this equilibrium is hampered favoring the ROS upsurge that culminates in oxidative stress [34]. Oxidative stress is through a series of events and its sustained presence may lead to pathogenesis of several ailments [35]. ROS readily attack and induce oxidative damage to various biomolecules including proteins, lipids, mitochondria, lipoproteins and DNA [36]. Oxidative stress affects many cellular functions by various mechanisms such as alteration in gene expression through activation of transcription factor NF-κB or induction of permeability transition in mitochondria with lethal consequences [37].

The data of the present study indicated that exacerbation of oxidative injury in liver and kidney tissues of PM-treated mice was more than control group as evidenced by elevation in LPO and reduction in GSH, CAT and SOD. These results corroborate with data from many previous researchers who reported similar results with different OPs in experimental animals [9, 12, 17, 30-32]. Different mechanisms have been postulated to explain PM-induced liver and renal injury, such as lipid peroxidation and interaction with membrane components. In fact, lipid peroxidation represents one of the most frequent reactions resulting from free radicals’ attack on biological structures [9, 12, 30]. Glutathione is an important naturally occurring antioxidant, which prevents free radical damage and helps detoxification by conjugating with chemicals. In addition, GSH is central to the cellular antioxidant defenses and acts as an essential cofactor for antioxidant enzymes including glutathione peroxidase and glutathione S-tranferase [12, 33]. Under oxidative stress, GSH is consumed by GSH related enzymes to detoxify the peroxides produced due to increased lipid peroxidation [17, 30].

The liver is the main organ where exogenous chemicals are metabolized and eventually excreted. All the major liver functions can be detrimentally altered by acute or chronic exposure to toxins [38]. When toxicants, including OP insecticides inhibit or otherwise impede...
hepatic transport and synthetic processes, dysfunction can occur [38]. However, the response of liver to chemical exposure mainly depends on the intensity and duration of the insults. Our present study demonstrated that PM treatment significantly elevated serum levels of hepatic enzymes (ALT, AST and ALP), total protein and albumin level in a dose-dependent manner which are indicators of hepatocellular damage. The biochemical alterations as well as the histopathological findings in the present study such as focal hepatic necrosis, inflammatory cells infiltrate and Kupffer cells activation orchestrate together to describe the hepatotoxicity. Since necrosis has been characterized by cell swelling and leakage of the cellular contents into blood stream, the elevation of hepatic enzymes in the serum considered to be the biomarker of liver damage and dysfunction. These results are in consistent with many previous investigators with different OP insecticides [17, 30-33] who postulated that elevation of LPO in kidney and liver tissues is concomitant with destruction of cell wall integrity (the lipid, the main constituents of plasma membrane), which in turn facilitate the leakage of the cytosol to the blood stream. Similarly, the kidney is a vital organ and its main function is to maintain total body fluid volume, its composition and acid-base balance. Numbers of environmental variables including certain xenobiotics influence these functions [39]. The significant elevations observed in serum urea and creatinine accompanied by histopathological findings such as atrophy of glomerular tuft and perivascular edema which are indicators of renal dysfunction, among the PM-treated groups is consistent with previous documents on the OP-induced nephrotoxicity [12, 32, 33]. In fact, chemically induced increases in serum creatinine and/or urea may not necessarily reflect renal damage but rather may be secondary to dehydration, hypovolemia, and/or protein catabolism [12, 40]. Of course these alterations should be taken into consideration in evaluating urea and/or serum creatinine as potential end point of renal toxicity when correlate with renal histopathology.

In our study, there is a correlation between the histopathological findings and the alterations of serum liver and kidney biomarker functions. It has been suggested that the ability of PM to cause alterations in the liver and kidney biomarkers functions could be a secondary event following lipid peroxidation and oxidative damage.

In conclusion, this study revealed that pirimiphos-methyl insecticide induced hepato-renal toxicity in male mice manifested by significant alterations in body and relative organ weights, serum ALT, AST, ALP, total protein, albumin, urea and creatinine level as well as hepato-renal damage manifested by induction of lipid peroxidation and depletion in SOD, CAT and GSH activities in liver and kidney tissues of mice. However, the ultimately effects was observed in the highest dose (1/20 LD50). The intensity of the adverse effects in male mice exposed to different doses of PM mainly relies on the dose-dependent manner.

COMPETING INTERESTS
The authors declare that they have no conflict of interest.

REFERENCES

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