Effect of organophosphorus insecticide, chlorpyrifos and synthetic pyrethroid, deltamethrin at chronic period in liver and serum of albino mice, *Mus musculus*

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**ABSTRACT**

In this research we used chlorpyrifos (organophosphorus pesticides) and deltamethrin (synthetic pyrethroid pesticides) to study their oxidative stress on albino mice’s liver and serum. Those pesticides were given orally at $\frac{1}{100} LD_{50}$ repeatedly for ninety days to represent chronic treatment. Antioxidants enzymes SOD, CAT, MDA, GSH and GXP were monitored in treated and untreated mice. $LD_{50}$ Came to be 54 mg/kg and 9 mg/kg B.W. for chlorpyrifos and deltamethrin, respectively. Chlorpyrifos caused an oxidative stress on both liver tissues and serum as manifested in the above tested antioxidant enzymes. In liver tissues all enzymes were lower except SOD. Meanwhile, all enzymes in serum came to be lower except CAT and GXP showed higher values over the control. Deltamethrin chronic treatment caused an elevation in SOD and GXP in liver tissues and serum. At the same time CAT, MDA and GSH showed a lower values comparing to the control in both liver tissues and serum

**Keywords:** *Mus musculus*, organophosphate, synthetic pyrethroids, oxidative stress, liver, serum, chronic treatment.

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**INTRODUCTION**

Pesticides are compounds that commonly used to kill pests and control of insect vectors of disease to increase yield of several crops. However they caused severe environmental pollution and health hazard to non-targeted organisms. Organophosphorus compounds are most favorite insecticides due to their high toxic potential and low environmental persistence (Tripathi and Srivastav, 2010). Also the use of synthetic pyrethroids as insecticidal and anti-parasitic formulations has markedly increased in last two decades (Mestres and Mestres, 1992). Furthermore, Pesticides have been the cause of many severe acute and chronic human poisoning (Casida and Quistad, 1998 and Soderlund, 2000). Organophosphates mode of action depends on inhibitions of the function of carboxylic ester hydrolases such as butyrylcholinesterase (BCHE), chymotrypsin, acetylcholinesterase (ACHE), plasma and hepatic carboxylestrase and nonspecific esterases within the body (Soltaninejad and Abdollahi, 2009). Pyrethroids are axonic excitotoxins, the toxic effects of which are mediated through preventing the closure of the voltage-gated sodium channels in the axonal membranes. The sodium channel is a membrane protein with a hydrophilic interior. This interior is a tiny hole which is shaped precisely to strip away the partially charged water molecules from a sodium ion and create a favorable way for sodium ions to pass through the membrane, enter the axon, and propagate an action potential. When the toxin keeps the channels in their open state, the nerves cannot repolarize, leaving the axonal membrane permanently depolarized, thereby paralyzing the organism.
Oxidative stress is the disturbance in the equilibrium between prooxidant and antioxidant in cells. Most harmful effects of oxygen are due to formation of reactive oxygen species which donate oxygen to other substance. Many of them are free radicals. Free radicals are highly reactive and unstable due to presence of one or more unpaired electrons. Those play a dual role as deleterious and beneficial in the cells. They are derived from normal metabolism in human body or from external sources such as exposure to rays, industrial chemicals and pesticides (Razaie, 2007 and Abdollahi, 2004). We have to mention that choosing oral administration as it recommended as the most appropriate in long-term exposure of chlorpyrifos as it enters the animal’s body through the residues in food (Tripathi and Srivastav, 2010). It is become evident that pesticides may induce oxidative stress leading to generation of free radicals and alteration in antioxidants or oxygen free radicals scavenging enzyme system (Banerjee, 1999). The balance between the production of free radicals and antioxidants defenses in the body has important health implication if there are too many free radicals or too few antioxidants for protection, a condition of oxidative stress develop, which may cause chronic and permanent damage (Harman, 1998). Many compounds have been detected to have antioxidant activity. Antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase and vitamin C, vitamin E and beta-carotene are the most important antioxidant defense in biological systems (Hailiwell and Gutteridge, 1990) and (Sies and Stahl, 1998).

Putting the above facts in considerations we preformed this research to detect the ability of chronic oral administration of chlorpyrifos and deltamethrin to induce oxidative stress in mice’s liver and serum. Several enzymes that are known to reflect the presence of responses of antioxidant enzymes Superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), glutathione (GSH) and glutathione peroxidase (GPX) will be monitored.

**MATERIALS AND METHODS**

**A- MATERIAL**

**I- Pesticides**

We choose two pesticides in this study to explore their oxidative stress and anti-oxidative responses in mice. Those pesticides are first, organophosphorus compound which known to be toxic to the body and become an integrated part of the ecosystem. It has been used in domestic pest control and agriculture programs. It has high insecticidal activity, low environmental persistence and moderate toxicity. Therefore those compounds are most favored insecticides (Tripathi and Srivastav, 2010). Second chosen pesticide is deltamethrin which used in crop protection and control of malaria and other vector borne diseases (Barlow, 2000). In the following there are their chemical structure and names.

**a- Chlorpyrifos**

*Trade name:* Genpest – brodan, eradex, lorsban, piridane, sulban, deviban, chlorofet, dursban.

*Chemical structure:* O,O-Diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate.

![Chemical structure of Chlorpyrifos](image)

*Action:* insecticide.

*Use:* for alfalfa, aphids, armyworms, billbugs, stalk borer, corn borers, corn earworm, corn rootworm adults, peach tree borer, overwinter scale, seed corn maggot, soil application, fire ants, ornamental plant insects, stored product insects and wood destroying insects.

*Toxicity:* oral \( LD_{50} \) 96-270 mg/kg (rabbit)

*Formulation used:* 48% EC.

**b- Deltamethrin**

*Trade name:* Deltamethrin, butoflin, butox, vapochothin.
Chemical structure: (S)-α-cyano-m-phenoxybenzyl(1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate.

Action: insecticide.
Use: for flying, crawling insects, on garden crops, fruit crops, field crops, for coleoptera, homoptera and Lepidoptera, may be used alone except against mites.
Toxicity: oral $LD_{50}$ (rat) 128.5 mg/kg.
Formulation used: 5% EC.

2- Animals

Male albino mice weighing 25-30g are used. The animals were purchased from Competence Farm in Tanta and they were kept in our laboratory under standard conditions of temperature (25 ± 2) and light and they were provided with sufficient food and water. Clean plastic cages were used for this purpose.

B- METHODS

Pesticides were used as oral administration as it seems to be the most appropriate in long term exposure, as it enters the animal body through the residues of food (Tripathi and Srivastav, 2010). The toxicity lines were made using serial concentrations of both chlorpyrifos and deltamethrin. Each concentration was replicated seven times. Each replicate was represented by five mice. Calculated $LD_{50}$ values came to be 54 mg/kg B.W. and 9 mg/kg B.W. for chlorpyrifos and deltamethrin respectively. Animals were treated daily with $\frac{1}{100}LD_{50}$ of the tested pesticides for 90 days to represent the chronic treatment. At the same time a group of ten mice were given water only to represent the control (untreated). Several antioxidant enzymes were detected in liver and serum. Those enzymes are:

1- SOD assay superoxide dismutase according to (Nishikimi, 1972).
2- CAT assay catalase according to (Sinha, 1972).
3- MDA assay malondialdhyde according to (Esterbauer, 1982).
4- GSH assay glutathione according to (Habig, 1974).
5- GPX assay glutathione peroxidase according to (Habig, 1974)

All data were recorded as a mean number of 5 replicates for each treatment.

Statistical Analysis:
The obtained data were statistically analyzed using t-test (Petrie and Watson, 1999). Student’s t-test was used for determination of the level of significance of difference between different groups. The values were considered significantly at $P<0.05$. The statistical analysis was performed using SPSS.

RESULTS AND DISCUSSION

Figs (1) and (2) clear the toxicity of both chlorpyrifos and deltamethrin in the male albino mice orally treated and mortality percentages were recorded after 24 hrs. The results showed that the $LD_{50}$ values were 54 and 9 mg/kg B.W for chlorpyrifos and deltamethrin respectively.
Figure 1. Log probit toxicity line of chlorpyrifos after 24 hrs of oral treatment to albino mice male, *Mus musculus*

Figure 2. Log probit toxicity line of deltamethrin after 24 hrs of oral treatment to albino mice male, *Mus musculus*

Table 1. Effect of chlorpyrifos oral chronic treatment (90 days) on antioxidants in mice liver tissues.

<table>
<thead>
<tr>
<th>Antioxidants</th>
<th>Treated</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (m.mol/g)</td>
<td>454.00 ± 2.274496*</td>
<td>315.50 ± 4.38115</td>
</tr>
<tr>
<td>CAT (m.mol/g)</td>
<td>0.30 ± 0.000577*</td>
<td>0.32 ± 0.01453</td>
</tr>
<tr>
<td>MDA (n.mol/g)</td>
<td>2.79 ± 0.087623**</td>
<td>2.25 ± 0.02887</td>
</tr>
<tr>
<td>GSH (n.mol/g)</td>
<td>2.33 ± 0.146780**</td>
<td>4.63 ± 0.06386</td>
</tr>
<tr>
<td>GXP (m.mol/g)</td>
<td>21.54 ± 0.780285*</td>
<td>22.84 ± 1.26243</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SEM (n =5), student ‘t’ test.

* Significant difference at P<0.05 compared to the control.

ns=Not significant difference at P<0.05 compared to the control.

Whereas: Superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), glutathione (GSH) and glutathione peroxidase (GPX).

Data in table (1) clear the chronic effect of chlorpyrifos as antioxidants in liver of male’s mice. After 3 months of oral treatment. The amount of SOD in the liver tissues increased significantly comparing with the control. As for CAT, it decreased upon treatment with chlorpyrifos, with a significant differences with the untreated samples serving as the control. Both MDA and GSH, are different from the control. As the chronic oral treatment did not affect their amounts comparing with the control. In the control samples the MDA (n.mol/g) was 2.25 comparing with 2.79 n.mol/g in treated mice. The same result was obtained with GSH. GXP (m.mol/g), since its amount with chlorpyrifos treatment was 21.54 comparing with 22.84 for the control. These results agree with what (Wankhade, 2012) reported that liver marker enzymes (antioxidant enzymes) are adversely affected by chlorpyrifos intoxication in rats. According to Statistical Analysis, enzymes activities were significantly high (p < 0.05) except MDA and GSH were not significantly different (p = 0.983) and (p = 0.319), respectively.
Table 2. Effect of chlorpyrifos oral chronic treatment (90 days) on antioxidants in mice serum

<table>
<thead>
<tr>
<th>Antioxidants</th>
<th>Treated</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (m.mol/g)</td>
<td>34.10 ± 2.214347*</td>
<td>91.66 ± 0.59088</td>
</tr>
<tr>
<td>CAT (m.mol/g)</td>
<td>114.80 ± 2.378608*</td>
<td>100.00 ± 1.76383</td>
</tr>
<tr>
<td>MDA (n.mol/g)</td>
<td>2.09 ± 0.003512*</td>
<td>1.79 ± 0.00577</td>
</tr>
<tr>
<td>GSH (n.mol/g)</td>
<td>1.56 ± 0.283392**</td>
<td>2.45 ± 0.08677</td>
</tr>
<tr>
<td>GPX (m.mol/g)</td>
<td>21.27 ± 0.595007**</td>
<td>12.86 ± 0.32495</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SEM (n = 5), student ‘t’ test.
* Significant difference at P<0.05 compared to the control.
** Not significant difference at P<0.05 compared to the control.

Whereas: Superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), glutathione (GSH) and glutathione peroxidase (GPX).

Table 3. Effect of deltamethrin oral chronic treatment (90 days) on antioxidants in mice liver tissues.

<table>
<thead>
<tr>
<th>Antioxidants</th>
<th>Treated</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (m.mol/g)</td>
<td>490.00 ± 2.886751*</td>
<td>315.50 ± 4.38115</td>
</tr>
<tr>
<td>CAT (m.mol/g)</td>
<td>0.26 ± 0.008686*</td>
<td>0.32 ± 0.01453</td>
</tr>
<tr>
<td>MDA (n.mol/g)</td>
<td>2.70 ± 0.119304**</td>
<td>2.25 ± 0.02887</td>
</tr>
<tr>
<td>GSH (n.mol/g)</td>
<td>2.73 ± 0.260278**</td>
<td>4.63 ± 0.06386</td>
</tr>
<tr>
<td>GPX (m.mol/g)</td>
<td>35.88 ± 1.384164*</td>
<td>22.84 ± 1.26243</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SEM (n = 5), student ‘t’ test.
* Significant difference at P<0.05 compared to the control.
** Not significant difference at P<0.05 compared to the control.

Whereas: Superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), glutathione (GSH) and glutathione peroxidase (GPX).

Data in table (3) showed the response of deltamethrin treated mice liver tissues to induce antioxidants. Tested enzymes SOD, MDA and GPX showed a higher values over the untreated mice, as they showed 490, 2.7 and 35.88 compared to 315.5, 2.25 and 22.84 respectively. On the other hand CAT and GSH showed lower values in treated mice compared to untreated ones. They came to be 0.263 and 2.73 compared to 0.32 and 4.63 respectively. These results agree with what (Wankhade, 2012) reported that liver marker enzymes (antioxidant enzymes) are adversely affected by pesticides intoxication in rats. According to Statistical Analysis, enzymes activities were significantly high (p < 0.05) except GXP and GSH were not significantly different (p = 0.149) and (p = 0.994) respectively.

Table 4. Effect of deltamethrin oral chronic (90 days) treatment on antioxidants in mice serum

<table>
<thead>
<tr>
<th>Antioxidants</th>
<th>Treated</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (m.mol/g)</td>
<td>96.50 ± 0.681819*</td>
<td>91.66 ± 0.59088</td>
</tr>
<tr>
<td>CAT (m.mol/g)</td>
<td>81.50 ± 0.753510*</td>
<td>100.00 ± 1.76380</td>
</tr>
<tr>
<td>MDA (n.mol/g)</td>
<td>0.25 ± 0.060093*</td>
<td>1.79 ± 0.00577</td>
</tr>
<tr>
<td>GSH (n.mol/g)</td>
<td>0.15 ± 0.292138**</td>
<td>2.45 ± 0.08677</td>
</tr>
<tr>
<td>GPX (m.mol/g)</td>
<td>18.17 ± 1.721437**</td>
<td>12.86 ± 0.32495</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SEM (n = 5), student ‘t’ test.
* Significant difference at P<0.05 compared to the control.
** Not significant difference at P<0.05 compared to the control.

Whereas: Superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), glutathione (GSH) and glutathione peroxidase (GPX).

Data in table (4) represent the effect of deltamethrin oral chronic treatment on antioxidants in mice serum. It is clear that response of antioxidant value upon chronic treatment resemble the same response appeared in subchronic treatment in table (16). Enzymes SOD, MDA and GPX showed higher values in treated mice serum as they showed 96.5, 2.55 and 18.17 compared to 91.66, 1.79 and 12.86 respectively. Moreover, enzymes CAT and GSH showed a decrease in values in deltamethrin oral chronic treatment compared to the control. Our previous obtained results coincide with what (Tuzmen, 2008) reported using the same
two pesticides on rats. They reported that deltamethrin and chlorpyrifos cause different responses in antioxidative defence mechanisms depending on pesticides treatment and doses in liver tissues. Furthermore, there is an agreement with what (Manna, 2005) published upon rat treatment with deltamethrin in liver tissues. According to Statistical Analysis, enzymes activities were significantly high (p < 0.05) except GSH and GXP were not significantly different (p = 0.994) and (p = 0.191) respectively.

Figure 3. Effect of deltamethrin and chlorpyrifos on SOD in both liver and serum after 90 days of oral treatment with $\frac{1}{100} LD_{50}$

Chronic treatment that lasted for 90 days exerted its effect on SOD antioxidant as presented in fig (3). It is clear that liver tissue showed higher values in deltamethrin and chlorpyrifos as they came to be 490 and 454 m.mol/g respectively. Whereas deltamethrin induced SOD in serum as three times equals to that produced upon chlorpyrifos treatment. They came to be 96.5 and 34.1 m.mol/ml respectively.
Regarding CAT antioxidants, there are very low values upon deltamethrin and chlorpyrifos in liver tissue. On the contrary the enzyme level was very high in serum upon deltamethrin and chlorpyrifos treatments. They registered 114.8 and 81.5 m.mol/ml respectively as they presented in fig (4).

Figure 4. Effect of deltamethrin and chlorpyrifos on CAT in both liver and serum after 90 days of oral treatment with $\frac{1}{100}LD_{50}$

Data in fig (5) showed the response of MDA antioxidants to deltamethrin and chlorpyrifos in serum and liver tissue. There were no much variation in the enzyme level regardless of used organs or lasted pesticides.

Figure 5. Effect of deltamethrin and chlorpyrifos on MDA in both liver and serum after 90 days of oral treatment with $\frac{1}{100}LD_{50}$

Figure 6. Effect of deltamethrin and chlorpyrifos on GSH in both liver and serum after 90 days of oral treatment with $\frac{1}{100}LD_{50}$
Concerning antioxidant GSH, our obtained data presented in fig (6). It is clear that the enzyme level came to be higher in liver tissue than in serum. There was no variation in its level in serum regardless on the used pesticide. Meanwhile, there was a higher level in liver tissue upon deltamethrin and chlorpyrifos treatment.

![GXP (90 days)](image)

Figure 7. Effect of deltamethrin and chlorpyrifos on GXP in both liver and serum after 90 days of oral treatment with \( \frac{1}{100} LD_{50} \)

Data presented in fig (7) clearly show the effect of deltamethrin and chlorpyrifos on induction of GXP antioxidants. Deltamethrin excreted higher stress in liver tissue than in serum causing production of higher level of the GXP antioxidant. However, there was no much variations in its level upon chlorpyrifos treatment. Increased the level of GSH may be due to its role of detoxification of organophosphate (Banerjee, 1999). The deleterious effect of used pesticides is much more serious in multiple exposure than that of single dose. The previous obtained data go nicely with what (Tuzmen, 2008) suggested that alteration in antioxidant enzymes may be due to involvement of free radicals intermediates upon chlorpyrifos and deltamethrin toxicities.

REFERENCES