TOXICOLOGICAL IMPACT OF CERTAIN EMULSIFABLE CONCENTRATES OF CHLORPYRIFOS ON MALE ALBINO RATS WITH REGARD TO THERAPEUTIC ROLE OF SELENIUM

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ABSTRACT

The present study aimed to investigate the toxic effects of three trade names of chlorpyrifos (CPF) pesticide from different local manufactures; i.e. chlorozan (K), pestpan (W) and pyriban (H) on haematological indices, hepatic oxidative stress, in addition to lipid profile and thyroid hormone status in plasma of male albino rats. Also, to assess the therapeutic role of selenium (Se) under these conditions. Three compounds (K, W, and H) were administrated orally to rats at 23.43, 21.40 and 17.43 mg/kg b.w., respectively. (which represent the ¼ LD50) with 5 doses per week for 28 days, and then these rats supplemented with Se at 50 μg/day (RDA-US) as a therapeutic agent for 15 days. Additional group received Se (50 μg/day) alone for the same time interval to assay whether Se has any biphasic effect. The results showed that CPF treatment (H), caused erythropenia, associated with decreasing of haemoglobin (Hb) concentration and packed cell volume (PCV) in rats. In contrast, the same treatment induced the leukocytosis and lymphocytosis. Also, Se-supplemented rats had leukocytosis and neutrophilia. However, supplemented rat with Se following treatment with CPF (H) improved the erythrogram, whereas leukopenia and lymphopenia occurred in rats received Se following treatment with CPF (K). Chlorpyrifos treatments (K, W and H groups) did not alter markedly the hepatic lipid peroxidation (LPO) levels, while, the induction in the hepatic total glutathione (GSH) was occurred, compared with control group. The similar results were recorded in Se-supplemented rats. However, the fluctuation in the activity of alanine aminotransferase (ALT) was detected in chlorpyrifos-treated rats (W and H), whereas the aspartate aminotransferase (AST) activity did not change markedly. Supplementation rats with Se following treatment with CPF (K, W and H) reduced the levels of LPO, compared with CPF-treated rats (K, W and H). This trend was more pronounced in Se-supplemented rats. In contrast, a significant enhancement in the activity of aminotransferases, i.e. ALT and AST was observed in rats supplemented with Se, after treatment with CPF (K and W). Meanwhile, a marked inhibition in the activities of ALT and AST was detected in Se-supplemented rats. Overall, hypertriglyceridemia and hypercholesterolemia were observed in rats whether treated with CPF alone or pre-supplementation with Se and also in Se-supplemented rats.

However, an elevation markedly in the thyroxine (T4) and triiodothyronine (T3) levels was found in CPF-treated rats (W and K, respectively). While a marked decrease in the level of T3 was detected in rat supplemented with Se post-treatment with CPF (K and H) or alone. Moreover, the concentration of Se in hepatic tissues of rats received the Se following treatment with CPF was in order of W>H>K groups, whereas the Se element did not detected in liver tissues of Se-supplemented rats.

Conclusion: Selenium supplementation to CPF-treated animals improved the haematological findings and hepatic lipid peroxidation level, in addition to lipidogram (i.e. HDL-C).

Key words: Chlorpyrifos; Oxidative stress, Haematological indices, Rats, Lipid profile, Thyroid hormones.
INTRODUCTION

Occupational exposure to pesticides is becoming a common and increasingly alarming worldwide phenomenon. The health effects caused by this occupational exposure to pesticides are enormous.

Chlorpyrifos (CPF) was introduced to the Egyptian market, since thirty years at least, recently over ten trade names are available in the market but most common used are the previous mentioned trade names, commonly used to control cotton pests.

Acute and chronic exposure to chlorpyrifos has resulted in considerable liver damage as evidenced by changes in aspartate transaminase (AST), and alanine transaminase (ALT) (Goel, et al., 2000). Both, organochlorines (OC) and Organophosphate (OPs) compounds have been reported to produce oxidative stress (Ciccheti and Argentini, 2003). Chlorpyrifos reported to produce oxidative stress resulting in accumulation of lipid peroxidation (LPO) products in different organs of rats (Verma and Srivastava, 2003 and Mahaboob and Siddiqui, 2001).

Selenium is an essential trace nutrient for humans and animals. The biological roles ascribed to selenium (Se) include the prevention of cancer (Combs and Lu, 2001), Cardiovascular disease (Rayman, 2002 and Beckett et al., 2004), and viral mutation (Beck, 2001) in addition to, selenium prevents liver necrosis (Hoekstra, 1975). Because of selenate is more stable and less toxic than selenite, therefore, selenate can be regarded an appropriate form of selenium supplementation (Gasallany et al., 1984), however, in vivo selenate is reduced to selenite, which in turn enters the main stream of metabolism and this process is not rapid, but takes several hours (Spallholz, 1994). Virtually, no available literatures concerning the protective ability of Se against the deteriorative effects of pesticides, therefore, the present study is an effort aimed:

* To investigate the adverse effects of three trade names of chlorpyrifos (CPF) on haematological parameters and oxidative stress, in addition to lipid profile and thyroid hormones status.
* To verify the role of selenium (Se), as a therapeutic agent, in overcoming the deleterious consequences, induced by chlorpyrifos treatment.
* To determine Se concentration in liver tissues following supplementation of Se at Recommended Daily Allowance (RDA-US) either in CPF-treated rats or normal rats dosed Se alone for 15 days.

MATERIALS AND METHODS

Materials:

Three trade names of Chlorpyrifos active ingredient, 48% EC., (an organophosphorus group), locally formulated in Egypt and were used in this study:

First: Trade name Chlorzan (K), obtained from Kafr El-Zyat Co.
Second: Trade name Pestban (W), obtained from El-Watania Co.
Third: Trade name Pyripin (H), obtained from El-Helb Co.

Preparation of dose:

Each emulsifiable concentrate was emulsified in water immediately before use and orally administrated into animals by esophageal intubation (per OS.).

The calculated median lethal dose (LD50) of three trade names of Chlorpyrifos; CPF-K, CPF-W and CPF-H were 93.75, 85.58 and 71.13 mg/Kg b.w., respectively, according to Weil’s method (Weil, 1952).

Sub-acute dose, represent ¼ LD50 for each emulsifiable concentrate was diluted in water and used for dosing through experimental period.

Sodium selenate dose (50 μg/day) which represents the Recommended Daily Allowance (RDA-USA) was calculated and prepared according to the method of Paget and Burns (1964).

Animals and chlorpyrifos treatments:

- Male albino rats weighting 150 ± 10 g obtained from the farm of General Organization of Serum and Vaccine (Helwan Farm), Egypt, were used for the study. The animals were housed in plastic cages and allowed to adjust to the new environment for a week before starting the experiment. Rats were fed standard food pellets and tap water ad libitum. The rats were housed at 23 ± 2 °C and in daily dark / light cycle.

Animals were randomly divided into four groups of twenty animals each as the following.

Group – C: animals served as control group and given tap water instead of pesticide in parallel to the treated group.
Group – K: animals were given CPF-K at 23.43 mg / Kg b.w.
Group – W: animals were given CPF-W at 21.40 mg / Kg b.w.
Group – H: animals were given CPF-H at 17.83 mg / Kg b.w.

Each rat in each group was given orally repeated dose of Chlorpyrifos over period of 28 days (5 doses / week). Clinical behaviors were monitored daily.

Clinical pathology:

At the end of the study period (28 days), Chlorpyrifos treatments were stopped and animals were fasted overnight; anesthetized by inhalation of diethyl ether and blood samples were drawn by puncturing the orbital sinus of the animals by
heparinized capillary tubes (Schalm, 1986) as the following;

1. Blood samples were put immediately into disposable glass tubes with heparin, as the anticoagulant and centrifuged at 3500 rpm for 15 min. to obtain the plasma, which were kept at -40 °C, tell the different assay were carried out using diagnostic kits (Table 1).

2. Other blood samples were taken in dry clean tubes containing Ethylene Diamine Tetraacetic Acid (EDTA) as anticoagulant (1 mg/1 ml blood) according to Schalm (1986), for further haematological indices (Table 1).

From each group, five rats were sacrificed and livers were removed immediately and washed in ice cold normal saline and dried with fold of filter paper. One lobe was preserved at -40°C for estimation of total glutathione (GSH) and lipid peroxidation (LPO) level (Table 1).

The thyroxine (T4) and triiodothyronine (T3) hormones were determined by radioimmunoassay method (RIA), as shown in Table 1.

Supplementation with selenium:

After the collection of blood and livers samples, the remaining CPF-treated animals supplemented with selenium (Se) in the form of sodium selenate at 50 μg/day, as a therapeutic agent for 15 days. At the same time, additional group of normal rats (10 rats) received orally RDA of Se for the same time interval (15 days) to evaluate the biphasic effect of selenium alone (group Se). At the end of selenium supplementation period (15 days), blood samples were collected from CPF-K + Se, CPF-W + Se and CPF-H+ Se as mentioned before. Furthermore, five rats were sacrificed and livers taken out immediately after dissection. One lobe was preserved at -40 °C for biochemical analysis (Table 1) and the other lobe was kept for determination of Se concentration in liver tissues.

**Determination of selenium concentration in liver tissues:**

Selenium analysis in liver tissues was preformed according to the procedure which reported in AOAC (2004), by using atomic absorption spectrophotometer (Thermo Jarell Ash-AA Scan1).

**Statistical analysis:**

The results are expressed as mean ± S.E.M. the statistical significant of the data has been determined using student’s ‘t’ test, (Snedecor and Cochran, 1967).

### Table (1): Procedures adopted for determination of biochemical, hormonal and haematological parameters:

<table>
<thead>
<tr>
<th>parameters</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine aminotransferase (ALT) (Plasma)</td>
<td>Reitman and Frankel (1957)</td>
</tr>
<tr>
<td>Aspartate aminotransferase (AST) (Plasma)</td>
<td>Reitman and Frankel (1957)</td>
</tr>
<tr>
<td>Total cholesterol (TCh) (Plasma)</td>
<td>Trinder (1969)</td>
</tr>
<tr>
<td>Triglycerides (TGs) (Plasma)</td>
<td>Buccalo and Davais (1975)</td>
</tr>
<tr>
<td>High density lipoprotein (HDL) (Plasma)</td>
<td>Warnick et al. (1983)</td>
</tr>
<tr>
<td>Total glutathione (GSH) (Liver)</td>
<td>Akerboom and Sies (1981)</td>
</tr>
<tr>
<td>Lipid peroxidation (LPO) (liver)</td>
<td>Ohkawa et al. (1979)</td>
</tr>
<tr>
<td>Thyroxine (T4) (Plasma)</td>
<td>Britton et al. (1975)</td>
</tr>
<tr>
<td>Triiodothyronine (T3) (Plasma)</td>
<td>Britton et al. (1975)</td>
</tr>
<tr>
<td>Haematological parameters (Whole blood)</td>
<td>Schalm (1986)</td>
</tr>
</tbody>
</table>

**RESULTS**

Table (2) Haematological findings in rats treated with chlorpyrifos (CPF) for 28 days alone and then supplemented these rats with selenium (Se) for 15 days as a therapeutic agent. Chlorpyrifos treatment (H), caused a significant decrease (P<0.01) in the erythrocyte counts (RBCs), haemoglobin concentration (Hb) (P<0.01) and packed cell volume (PCV) values (P<0.001) on day 28, when compared with control group.

No-significant differences in the erythrocytic measurements were observed in rats treated with CPF (K and W) as well as in rats supplemented only with selenium (Se) for 15 days compared with control group. The same trend was detected in chlorpyrifos-treated rats (K), pre-supplementation with Se, in compared with chlorpyrifos-treated rats (K).

Meanwhile, a significant decrease in the Hb concentration (P<0.05) by 17% and PCV values (P<0.001) by 25% and mean corpuscular volume (MCV) (P<0.05) by 22% was observed in rats.
supplemented with Se, post-treatment with CPF (W), compared with CPF treated rats (W). In contrast, there was a significant increase (P<0.001) in the RBCs count by 42%, Hb concentration by 41% and PCV by 39% in rats received Se after treatment with chlorpyrifos (H), compared with chlorpyrifos-treated rats (H).

However, insignificant changes in the total leukocyte counts (WBCs) were observed in chlorpyrifos-treated rats (K and W), whereas a significant increase in the WBCs and lymphocytes leukocytic count was noted in chlorpyrifos-treated rats (H), in compared with control group.

Overall, our results showed that chlorpyrifos (H) is more toxic to erythrocyte indices (i.e., RBCs count, Hb concentration and PCV values) in compared with control group.

In addition, Supplementation rats with Se post-treatment with chlorpyrifos (K), caused a marked decrease (P<0.01 and P<0.001, respectively) in WBCs counts and lymphocytes leukocytic count by 57% and 60%, respectively, compared with the chlorpyrifos-treated rats (K). Also, a remarkable reduction (P<0.01 and P<0.05) respectively in WBCs by 40% and lymphocytes leukocytic count by 44% was noticed in chlorpyrifos-treated rats (H), prior to supplementation with Se compared with rats administrated with chlorpyrifos alone.

Table (2): Effects of selenium on haematological indices in rats treated with sub-acute dose of certain emulsifiable concentrates of chlorpyrifos for 28 days.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Chlorpyrifos</th>
<th>Se</th>
<th>Chlorpyrifos + Se</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K</td>
<td>W</td>
<td>H</td>
<td>K</td>
</tr>
<tr>
<td>RBCs X10⁶/μL</td>
<td>6.216 ± 0.369</td>
<td>6.360 ± 0.232</td>
<td>7.002 ± 0.287</td>
<td>±</td>
</tr>
<tr>
<td>Hb g/dL</td>
<td>12.208 ± 0.587</td>
<td>13.313 ± 0.447</td>
<td>15.194 ± 0.670</td>
<td>±</td>
</tr>
<tr>
<td>PCV %</td>
<td>40.239 ± 2.663</td>
<td>43.40 ± 1.122</td>
<td>46.200 ± 0.800</td>
<td>±</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>66.239 ± 2.663</td>
<td>68.471 ± 2.174</td>
<td>66.500 ± 3.280</td>
<td>68.281 ± 4.819</td>
</tr>
<tr>
<td>MCHC %</td>
<td>32.765 ± 1.944</td>
<td>30.823 ± 1.686</td>
<td>32.836 ± 0.965</td>
<td>32.836 ± 0.660</td>
</tr>
<tr>
<td>WBCs X10⁶/μL</td>
<td>10.188 ± 0.583</td>
<td>12.490 ± 1.013</td>
<td>13.840 ± 2.708</td>
<td>14.950 ± 1.419**</td>
</tr>
<tr>
<td>Lymphocytes X10⁶/μL</td>
<td>8.084 ± 0.973</td>
<td>9.415 ± 0.633</td>
<td>10.040 ± 0.231</td>
<td>12.774 ± 1.735**</td>
</tr>
<tr>
<td>Neutrophils X10⁶/μL</td>
<td>1.732 ± 0.297</td>
<td>2.134 ± 0.460</td>
<td>2.712 ± 0.990</td>
<td>1.999 ± 0.172</td>
</tr>
<tr>
<td>Eosinophils X10⁶/μL</td>
<td>0.340 ± 0.103</td>
<td>0.120 ± 0.037</td>
<td>0.430 ± 0.251</td>
<td>0.152 ± 0.107</td>
</tr>
</tbody>
</table>

The data expressed as mean ± S.E.M for five animals.

*** = P<0.01 in comparison with control group, * = P<0.05 in comparison with control group,

*** = P<0.001 in comparison with control group, *** = P<0.01 in comparison with chlorpyrifos treatment group.

**a = P<0.01 in comparison with control group, *a = P<0.05 in comparison with chlorpyrifos treatment group.

K = Chlorpyrifos (Chlorzan), obtained from Kafr El-Zyat Co.

W = Chlorpyrifos (Pestban), obtained from El-Watania Co.
The influence of sub-acute toxicity of three trade names of chlorpyrifos (K, W and H) and Se administration on hepatic lipids peroxidation (LPO) and total glutathione (GSH) levels, in addition to lipid profile are shown in Table (3). Treatment with chlorpyrifos (K, W and H) showed no significant differences in hepatic lipid peroxidation (LPO) as measured by the amount of malondialdehyde (MDA) formed, compared with the control group, although LPO levels were increased above the values of control group (not significantly). In addition, all chlorpyrifos treatments caused a significant elevation (P<0.05, P<0.001 and P<0.01, respectively) in the levels of hepatic total glutathione (GSH), compared with the control group.

However, Se treatment to the normal rats resulted in a significant reduction (P<0.05) in the level of LPO by 18%, whereas, an elevation markedly (P<0.001) in the total glutathione (GSH) by 34% was occurred, in compared with the control group.

Also, Se treatment to chlorpyrifos-treated animals, led to a decrease of LPO by 15% in group (K), 13% in group W and 14% in group (H), compared with the three chlorpyrifos-treated groups.

Moreover, Se supplementation to chlorpyrifos-treated animals (H and W) resulted in a significant decrease (P<0.05 and P<0.01, respectively) in GSH levels by 32% and 27%, compared with chlorpyrifos-treated rats (H and W) but these values fall within the normal limits of control group.

The liver damage was evaluated by the measurements of plasma aminotransferases, i.e., alanine and aspartate aminotransferases (ALT and AST). The extent of liver damage sustained following exposure to chlorpyrifos (K, W and H) is shown in Table (3). No-significant differences between the control group and chlorpyrifos-treated groups in the activity of ALT and AST, except a significant increase (P<0.05) as well as a decrease in the ALT activity were observed in chlorpyrifos-treated animals (W and H groups, respectively).

In contrast, Se treatment to chlorpyrifos-intoxicated animals resulted in a significant increase in the activity of ALT enzyme. It was significantly increased by 11% (P<0.05), 16% (P<0.001) and 25% (P<0.05) in chlorpyrifos treatments groups, i.e, K, W and H, respectively.

Also, a marked elevation by 19 and 18% in the AST activity was observed in group K+Se (P<0.001) and group W+Se (P<0.05), as compared to chlorpyrifos-intoxicated rats (K and W).

As shown in Table (3), Se-supplemented rats had a significant decrease in the activities of ALT and AST (P<0.01 and P<0.05, respectively) by 20% and 13% respectively, as compared to control group.

Table (3): Effects of selenium on oxidative stress and lipid profile in rats treated with subacute dose of chlorpyrifos for 28 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Chlorpyrifos</th>
<th>Se</th>
<th>Chlorpyrifos + Se</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K</td>
<td>W</td>
<td>H</td>
<td>K</td>
</tr>
<tr>
<td>GSH µmol g⁻¹ wet w</td>
<td>9.404 ± 0.352</td>
<td>11.765 ± 0.568**</td>
<td>13.777 ± 0.151***</td>
<td>12.771 ± 0.360**</td>
</tr>
<tr>
<td>ALT U/ L</td>
<td>62.820 ± 2.879</td>
<td>66.209 ± 1.538</td>
<td>72.127 ± 1.793**</td>
<td>50.130 ± 3.061**</td>
</tr>
<tr>
<td>AST U/ L</td>
<td>185.163 ± 8.126</td>
<td>204.46 ± 2.990</td>
<td>178.48 ± 13.44</td>
<td>177.48 ± 9.47</td>
</tr>
<tr>
<td>TG mg/dl</td>
<td>30.710 ± 1.218</td>
<td>42.966 ± 1.985***</td>
<td>43.475 ± 1.834***</td>
<td>32.949 ± 1.063</td>
</tr>
<tr>
<td>TC Chol mg/dl</td>
<td>62.240 ± 4.409</td>
<td>61.772 ± 6.396</td>
<td>63.080 ± 8.060</td>
<td>82.278 ± 3.392***</td>
</tr>
<tr>
<td>HDL-C mg/dl</td>
<td>46.100 ± 1.813</td>
<td>32.733 ± 2.299***</td>
<td>38.791 ± 0.656***</td>
<td>43.041 ± 2.851</td>
</tr>
</tbody>
</table>

The data expressed as mean ± S.E.M for five animals.

* = P<0.05 in comparison with control group,
** = P<0.01 in comparison with control group
*** = P<0.001 in comparison with control group,
A significant elevation (P<0.001) in concentration of TGs was observed following chlorpyrifos treatment in groups K and W. Meanwhile, animals in group (H) did not exhibit any change in Triglycerides (TGs) levels, in comparison to the control group. Rats supplemented with Se alone for 15 days, had a marked elevation (P<0.001) in the TGs level by 62%, compared with the control group (as shown in Table 3).

Also, chlorpyrifos-intoxicated rats, prior to Se treatment responded by a significant elevation (P<0.001) in the TGs concentration in groups (K) and (H) by 28 and 38%, respectively.

Concerning with total cholesterol (TCh) levels, an administration of rats with chlorpyrifos did not induce any significant change, except in rats group (H), a marked elevation (P<0.01) in the TCh was occurred. However, the TCh levels did not alter markedly in rats supplemented with Se following treatment with chlorpyrifos, except in group (K), a marked elevation (P<0.001) in the level of TCh by 48% was observed. Moreover, levels of high density lipoproteins-cholesterol (HDL-C) were also found to be decreased markedly (P<0.01) in chlorpyrifos-treated animals in group K and in group W, as well as in Se-treated animals (P<0.001) by 42%. Supplementation with Se to chlorpyrifos-treated animals led to a significant increase of HDL-C by 37% (P<0.001) in group K, 26% (P< 0.001) in group W and 25% (P<0.05) in group H. Our results showed that all formulations of chlorpyrifos (K, W and H) had subtle effect on lipid peroxidation status, (LPO), but chlorpyrifos (K) was more obvious toxicologic effect on hepatocytes (LPO). Also, our data proved that chlorpyrifos (K and W) had a marked effect (i.e., a decrease) on plasma HDL-C level, rather than chlorpyrifos (H), (Table 3).

A significant elevation (P<0.05) in the levels of thyroxine (T4) and triiodothyronine (T3) in chlorpyrifos treated rats (W and K groups, respectively) were occurred. In contrast, a marked decrease (P<0.001) in the level of T4 by 32% was observed in rats supplemented with Se, following treatment with chlorpyrifos (w). Similar trend was noticed in the level of T3 (P<0.001), in rats whether supplemented only with Se for 15 days or following treatment with chlorpyrifos (K and H) by 99.7, 50 and 22%, respectively (Table 4).

The selenium (Se) element was detected in hepatic tissues of rats supplemented with Se, post-treated with CPF (K, W and H) for 28 days, where the values of Se were 78.09, 178.90 and 85.5 μg g⁻¹, respectively. Meanwhile the Se not detected in hepatic tissues of rats supplemented only with Se for 15 days, (as shown in Table 5).

| Table (4): Effects of selenium on thyroid hormones status in rats treated with subacute dose of chlorpyrifos for 28 days |
| --- | --- | --- | --- |
| Parameter | Control | Chlorpyrifos | Chlorpyrifos + Se |
| | | K | W | H | Se | K | W | H |
| Thyroxine μg/dl | 5.717 ± 0.440 | 6.487 ± 0.346 | 7.472 ± 0.247** | 5.760 ± 0.316 | 5.260 ± 0.060 | 5.727 ± 0.707 | 5.044 ± 0.354*** | 5.296 ± 0.150 |
| Triiodothyronine μg/dl | 0.225 ± 0.004 | 0.285 ± 0.014** | 0.213 ± 0.008 | 0.211 ± 0.007 | 0.070 ± 0.002**** | 0.146 ± 0.009*** | 0.188 ± 0.009 | 0.164 ± 0.002**** |

**a** = P<0.05 in comparison with control group,
***b** = P<0.001 in comparison with chlorpyrifos treatment group.

| Table 5: Levels of selenium (μg g⁻¹ Liver) in hepatic tissues of rats supplemented only with Se or post-treatment with chlorpyrifos. |
| --- | --- | --- |
| Normal rats + Se | Chlorpyrifos + Se |
| K | W | H |
| N.D | 78.09 | 178.90 | 85.53 |

Results are means of three animals
N.D = None-detected
DISCUSSION

There is evidence that many chemical agents and drugs known to be toxic to cells, which associated with the generation of free radicals, this may be due to simultaneously increased oxidative stress in the cells and decreased oxidative defense potential.

Our results suggest that erythropenia (decrease red cell mass), accompanied by decreasing of Hb concentration and PCV values, which occurred as response to chlorpyrifos treatment (H). This may be due to destruction or lysis of RBCs, as a consequence cells and decreased oxidative defense potential. In contrast, leukocytosis and lymphocytosis were detected in chlorpyrifos-treated rats (H). The secretion of epinephrine actually stressed animals, due to destruction or lysis of RBCs, as a consequence cells and decreased oxidative defense potential. This may be understood in view of the fact that Ops pesticides consume GSH through GST catalyzed reaction as a major way of detoxification and these chemical expected to induce the biosynthesis of GSH as a potent protection and reflect an activation of the compensatory mechanism through the effect of pesticides on progenitory cells and its extent depend on the magnitude of the oxidative stress and hence on the dose of stressor (Praksam, et al., 2001).

Lipid peroxidation is an oxidative deteriorative process of unsaturated fatty acids, due to excess generative of free radical. Our results suggest but don’t prove that free-radical mediated lipid peroxidation, may be involved in toxic manifestation of chlorpyrifos. It has previously reported that acute exposure to chlorpyrifos pesticides may induce oxidative stress in rat i.e. Khan and Kour (2007), Verma, et al. (2007) and Goel, et al. (2005), where, alteration in GSH, total thiols, and lipid peroxidation have been noted. Similar results were reported by Bebe and Panemangalore (2003) who mentioned that after endosulfan (OCH) exposure liver GSH level significantly increased by 25 and 40% in rats exposed to 4 and 6 mg / 250 g body weight. The normalization of LPO and a marked reduced in the glutathione level was observed following Se treatment to chlorpyrifos-treated rats compared with chlorpyrifos-treated rats. This is likely due to its antiperoxidative properties, irrespective of liver injury, and also this may be due to activation of glutathione dependent–enzymes, i.e., selenoenzymes (GPxs) (Chu et al., 1996). This shown by a significant reduction in the level of lipid peroxidation by 15, 13 and 14% in groups K, W and H, respectively.

Similarly, Se supplementation to normal rats decreased LPO by 18% and caused an induction of the GSH levels by 34% compared with control group. In the present study, there was insignificant differences in aminotransferases activities (ALT, AST) between the control group and the groups of animals treated with chlorpyrifos, except a significant increase (P<0.05) and a decrease (P<0.05) was noted in the ALT activity in chlorpyrifos-treated rats (groups W and H, respectively).

We have previously shown that chlorpyrifos intoxication alters serum and hepatic activities of antioxidant (i.e. GSH) at the gene might therefore take place.

Oxidants such as hydrogen peroxide (H2O2) activate specific gene expression through the antioxidant responsive elements (Rushmore, et al., 1991). Also, elevation of the glutathione level in the liver, may help to preserve physiological integrity of the liver. These findings are in line with the previous work, where an elevation in the levels of GSH in cells was observed following administration of N-acetyl cysteine or thioacetic acid (Iwata, et al., 1994). This can be understood in view of the fact that Ops pesticides consume GSH through GST catalyzed reaction as a major way of detoxification and these chemical expected to induce the biosynthesis of GSH as a potent protection and reflect an activation of the compensatory mechanism through the effect of pesticides on progenitory cells and its extent depend on the magnitude of the oxidative stress and hence on the dose of stressor (Praksam, et al., 2001).
liver marker enzymes including AST and ALT (Goel, et al., 2000 and Khan and Kour, 2007). It has been previously reported that during liver damage there is an observed decrease in antioxidant defense in the liver (Seven, et al., 2004). An enhancement of ALT activity, may be due to leakage of this enzyme from hepatocytes, however, a fall of ALT activity may be attributed to depletion of this enzyme and/or reduction in the number of cells rather than to recovery (Duncan, et al., 1994).

In contrast, a marked enhancement in the activities of ALT and AST were detected in rats supplemented with Se post-treatment with chlorpyrifos, where the ALT activity increased by 11, 16, and 25% in groups K, W and H, respectively, as well as the AST activity was increased by 19 and 18% in groups K and W, respectively, compared with chlorpyrifos-treated rats. This may be attributed to induction of these enzymes as a consequence of releasing of corticosteroids (stress hormones) (Duncan, et al., 1994).

Watabe, et al., (1999) reported that Se-deficiency caused a marked decrease in steroid hormones production. However, the administration of Se to normal rats, led to a marked inhibition in the activities of ALT and AST by 21 and 13%, respectively, compared with the control group. This may be attributed to an increase in the clearance rate of such enzymes in plasma of Se- supplemented rats (Zilva, et al., 1988).

With regard to the chlorpyrifos-treated animals, hypertriglyceridemia was observed in group k (P<0.001) and group W (P<0.01). Triglyceridemia a good evidence of increased hepatic glyceride synthesis, since there is evidence that the rate of synthesis is directly proportional to the concentration of the substrates present (Fatty acid and glycerophosphate) (Ruckebush et al., 1988). However, this may be due to increase the level of very low density lipoproteins (VLDL) which involved in transport of hepatic triglycerides to extra-hepatic tissues (Bartley, 1989).

Only, Hypercholesterolemia was noted in chlorpyrifos- intoxicated rats (group H). This could be a result of suppression the rate limiting enzyme, i.e., Hydroxy-3-methylglutaryl-Co A reductase (HMG-Co A reductase) and thus a new cholesterol synthesis occurred (Mayes, 1993). In the present study, we have observed low level of HDL-C following 28 days of toxicity by chlorpyrifos (groups K and W), this may be due to impairment of HDL-C synthesis. Cou-Dray, et al., (1997) reported that thioobarbituric acid reactive substances (TBARS) level was positively correlated with cholesterol and negatively correlated with triglycerides (TGs).

Administration of Se to the normal rats as well as to the chlorpyrifos-treated rats resulted in a marked elevation in the TGs, which is suggestive of its property to induce VLDL synthesis and that often associated with hyperinsulinemia, which may be a cause of the over production (Mayes, 1993). Also, it has been proved that Se has insulin-mimetic properties in vivo (Mc Neil, et al., 1991).

However, TCh increased by 48% only in group K as well as HDL-C level were increased significantly by 35, 26, and 25% in group K, W and H respectively, in comparison with chlorpyrifos-treated rats.

An inhibition of hepatic microsomal 7-hydroxylation of cholesterol by pesticide leads to reduction of TCh. breakdown and its accumulation (Varshney, et al., 1986) HDL-appear to be important in cholesterol efflux from tissues, thereby reducing the amount of cholesterol stored there (Stein, 1986). Also, HDL is involved in returning cholesterol from peripheral to the liver or removal as bile acid, a process known as reverse cholesterol transport (Mayes, 1993)

Elevated HDL concentration, as shown in our results, might has been correlated with a reduced risk for coronary artery disease (CAD), where HDL particularly attenuates the initiation and progression of atherosclerotic lesions (Stein, 1986). The current study demonstrated the alteration in lipid profile upon administration of three products of chlorpyrifos, since treatments increased TGs (Groups K and W) and TCh (Group H) and decreased HDL (Groups K and W). These results are in parallel with that of El-Kashoury and El-Far (2004) and El-Kashoury, et al. (2006), who recorded that profenfos (OP) increased TGs, TCh and decreased HDL in rats.

The present results suggest that chlorpyrifos treatment caused a marked increase in the thyroxine (T4) in group W and triiodothyronine (T3) in group K at the end of treatment (28 days).

Our findings are in agreement with some earlier observations with OP pesticide by El-Kashoury and El-Far (2004) and El-Kashoury, et al. (2006), who found that oral administration of profenfos (OP) into male albino rats induced a marked elevation in T3 and T4 levels, respectively.

This increment in circulating pool of T4 could be ascribed to the reduction of the metabolic clearance rate of T4, moreover, T4 has half life which is seven folds longer than that of T3 (Jameson and De Groot, 1995). However, an elevation in T3 level reported in the present study may be due to decreased utilization it in the different cells (Kaneko, 1997).
Meanwhile, our findings indicated that Se supplementation to normal rats or to rats treated with chlorpyrifos showed a significant decrease (P<0.001) in the level of plasma T3. Also, a marked decrease in the level of T4 was only observed in rats supplemented with Se, post-treated with chlorpyrifos (W). This may be due to increase the activity of selenodeiodinase i.e., 5'-DI. Selenium, in the form of selenocystein, functions as a catalytic center in the active site of triiodothyronine deiodinases involved in thyroid hormones metabolism (Salvatore et al., 1996).

The deiodinases have differing substrates specificities and tissue distribution (Bianco et al., 2002). These enzymes can catalyze the removal of iodine from the 5 or 5' positions of iodothyronine substrates (Chanione et al., 1993).

The selenodeiodinase control the metabolism of thyroid hormones, a deficiency in T3 level following treatment with Se, possibly due to increased expression of hepatic 5'-DI which providing an important source of plasma T3, and degrading rT3 and T3 sulphate, that is substrates for 5-deiodination by D1.

The depletion of Se in hepatic tissues of rats received the Se alone for 15 days was detected, while the Se accumulated in hepatic tissues of rats supplemented with Se, post-treated with chlorpyrifos for 28 days. This could be attributed to increasing consumption of Se and this led to a higher Se content of proteins in the form of selenomethionine, which may compete with sulfur in the biosynthesis of methionine, which is the precursor of GSH synthesis. However Se-plasma proteins (SPP) containing up to 70% of plasma Se and it secreted mainly from hepatic tissues (Mostert, 2000).

A general observation during depletion was the retention or redistribution of Se to the brain, the endocrine organs and the reproductive organs, whereas liver, muscle, skin and other large tissues rapidly lose their Se (Behne et al., 1988).

In conclusion, the results of this study showed that chlorpyrifos (H) had haematotoxicity compared with chlorpyrifos (W and K). While, chlorpyrifos (K) exerted highly extent in the LPO level and decrease HDL-C concentration rather than other formulations (W and H). Moreover, chlorpyrifos (K and W) altered the thyroid hormones levels.

Administration of Se to chlorpyrifos-treated rats exerts significant therapeutic activity against oxidative stress and lipid disorder (i.e., HDL-C), in spite of Se dosing depressed the level of T3, therefore, further studies are needed for better understanding of the causes of thyroid hormones disorders.

REFERENCES


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