Biochemical role of fermented wheat germ on liver and kidney functions alteration induced by chlorpyrifos in rats

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ABSTRACT
Chloropyrifos (CPF) is an organophosphate insecticide is widely used for a variety of agricultural and public health applications. The purpose of this study was to assess the biochemical role of the fermented wheat germ (avemar) on the liver and kidney function tests and the oxidative stress induced by chlorpyrifos in rats; moreover the heamatological measurements and histological investigation were studied. Chloropyrifos was added to the different experimental tested diets at two levels of low and high doses (25 and 50 mg/kg diet, respectively). The fermented wheat germ was added at a level of 3g/kg diet. The results demonstrated that there were significant decrease in the total counts of RBC’s, WBC’s, erythrocyte indices, hemoglobin concentration and hematocrit level in experimental rats fed diets containing low and high levels of CPF. Liver functions is impaired in rats administrated only chlorpyrifos either low or high dose and the results showed a significant increase in enzyme activities such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (γGT), while total proteins, albumin, and globulin showed a significant decrease at high and low doses of CPF treated groups but kidney functions results showed a significant increase in serum creatinine and urea levels. Administration of CPF caused a significant increase in lipid peroxidation level, lipid profile while the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione-s-transferase (GST) were decreased significantly. So avemar supplementation caused significant improvement in all results in comparison with those groups administrated CPF.

Key words: wheat germ, chlorpyrifos, liver functions, kidney functions, lipid peroxidation.

INTRODUCTION
Chloropyrifos (O, O-diethyl-O-(3, 5, 6-trichloro-2-pyridyl) -phosphorothioate) is a broad-spectrum organophosphate insecticide which is widely used in agriculture and in domestic use against harmful insects (Saulsbury et al., 2009). Liver is the organ where activation and detoxification of CPF takes place, while it is eliminated primarily through the kidney (Betancourt and Carr, 2004). Besides being potent anticholinesterase compound, CPF elicits a number of additional effects, including hepatic dysfunction, heamatological and immunological abnormalities, embroyotoxicity, genotoxicity, neurotoxicity and neuro-behavioral changes (Mehta et al., 2009). Many insecticides are hydrophobic molecules that bind extensively to biological membranes, especially phospholipids bilayers (Ogutcu et al., 2008), and they may damage membranes by inducing lipid peroxidation (LPO) (Kalender et al., 2010).

Wheat germ is the component of wheat kernel with the highest nutritional value (Zhu et al., 2006). The germ makes...
up only 2%-3% of the wheat kernel and is the most nutritious part of the wheat kernel. Nutrients are concentrated in the germ, and it is rich in vitamins, minerals, protein, and fats. Wheat germ contains high levels of tocopherol and B vitamins. It is separated from the other wheat components by the milling process.

In addition to the nutrients listed above, wheat germ can be subjected to fermentation with *Saccharomyces cerevisiae* to yield the benzoquinones 2, 6-dimethoxybenzoquinone (DMBQ) and 2-methoxybenzoquinone. These benzoquinones are present in unfermented wheat germ as glycosides; yeast glycosides activity present during fermentation leads to release of the benzoquinones as a glycones. The wheat germ fermentation end-product, which is suitable for human consumption, is a dried extract standardized to contain methoxy-substituted benzoquinones (2-methoxy-benzoquinone and 2, 6-DMBQ) at a concentration of 0.04% (Philipp et al., 2007). The fermentation process of wheat germ increases the nutritional and therapeutic properties of wheat germ. In the case of avemar fermentation produces biologically active quinones, which may be active principles. Quinones are carbonyl group molecules with a wide range of biological activity. One of their properties is to be able to attract and accumulate electrons in the carbon-hydrogen double bond. Benzo- and hydroquinones have antimicrobial activity. All of these compounds may modulate the immune system. The mechanism they share in common is suggested to be a free radical scavenging capability (Kenner, 2009).

The present study aimed to investigate the biochemical effects of low and high doses of chlorpyrifos in experimental animals and the effect of avemar supplementation in alleviation of the toxic effects of chlorpyrifos in rats.

**MATERIALS AND METHODS**

**Experimental animals:**

The healthy experimental animals used throughout the present work were 50 adult male albino Sprague-Dawely strains mean weight varied between 98g to 117g. They were obtained from El-Salam-Farm, Giza, Egypt. The animals were divided into 5 homogenous groups and housed individually in plastic cages fitted with a wire mesh bottoms and fronts in a room maintained at 25-30 °C with about 50% relative humidity. The room was lighted on a daily photo period of 12/12 hrs light / dark cycle. Then, they were allocated to the various experimental diets for 30 days.

**Experimental Design:**

The animals were divided into nine groups of rats (eight for each group). All rats offered a balanced diet prepared according to Reeves et al., (1993) for seven days for adaptation on the environmental conditions before starting the experiment. The experimental groups were fed on the balanced diet as control as well as the other tested groups fed on contaminated balanced diets with chlorpyrifos (CPF) at the two levels (25 and 50 mg/kg diet) or supplemented with treatment doses of avemar and plus the different doses of CPF, as follows:

- **Group (1)** received normal balanced diet (control).
- **Group (2)** received normal balanced diet plus low dose (25 mg/kg diet) of CPF.
- **Group (3)** received normal balanced diet plus high dose (50 mg/kg diet) of CPF.
- **Group (4)** received normal balanced diet plus of CPF, supplemented with avemar (3 g/kg diet).
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Group (5) received normal balanced diet plus high dose of CPF, supplemented with avemar (3 g/kg diet).

During the conditioning period and through out the experiment, food and water were provided *ad libitum*. At the end of the experimental period, the animals were fasted for 12 hrs, and then anesthetized under diethyl ether and whole blood samples were taken from hepatic portal vein in three centrifuge tubes. The first tube contained ethylene diamine tetraacetic acid (EDTA) was used for haematological analysis. The second tube contained heparin then centrifuged for 10 minutes at 4000 rpm and plasma kept in plastic vials at -20 °C till used for the biochemical analysis. The third tube were left for 15 minutes at 37°C then centrifuged at 4000 rpm for 20 minutes for separating serum, and then serum were removed and kept in plastic vials at –20 °C until analysis.

Liver and kidney were separated, rinsed and washed by saline solution (NaCl 0.9%), then blotted on filter paper, weighed and calculated their relative weights. The livers were stored in 10% formalin saline 50% v/v for 24 hrs until microscopical examination is done.

**Biochemical measurements:**

1- Hematological measurements of cellular fractions such as red blood cells (RBC'S), white blood cells (WBC'S), and platelets counts as well as erythrocyte indices, the constants depend on either erythrocyte size or hemoglobin content also determinations of blood hemoglobin and hematocrit levels.

2- Evaluation of some organ functions such as liver by determining some enzyme activities such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (γGT), as well as total proteins, albumin, globulins contents; A/G ratio and kidney function as measurements of creatinine and urea levels in serum.

3- Evaluation of some lipids peroxidation product as malondialdehyde and some antioxidant enzymes as superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST).

4- Determination of lipid profile in serum, as total lipids, total cholesterol, triacylglycerols, in addition to high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C) and LDL/HDL ratio.

5- Microscopical investigation will be carried out for normal and different experimental groups. Liver were dissected out and fixed instantaneously in 10% formalin saline 50% v/v for 24 hrs. The specimens were washed in tap water, dehydrated in ascending grades of ethanol, cleared in xylene, embedded in paraffin wax at melting point 55-60 °C. Sections of 6µm thickness were prepared and stained with haematoxylin and eosin Harris (1990).

**Statistical analysis:-**

Statistical analysis was done by using SPSS 11.5 statistical software completely randomization design in factorial arrangement (ANOVA; F-test) and one way classification to determine least significant difference (L.S.D) (Dawson and Trapp, 2004).
RESULTS

1. The effects of different experimental tested diets on red blood cells (RBC's), white blood cells (WBC's), platelets counts and hemoglobin, (Hb) hematocrit (Hct) values:

Table (1) shows significant decreases between values of RBC's, WBC's, platelets counts and Hb and Hct values of control group and values of untreated groups G2 and G3. The increment of RBC's in treated groups fed on low CPF were 46.93% for G4 when compared to G2 and also 89.23%, for treated group G5 when compared to G3. The results of WBC's, Hb, Hct and platelets showed an increment in G4 and G5 when compared with G2 and G3, respectively. The avermar enriched diet caused successful treatment for the defect that caused by CPF in RBC's, WBC's, Hb, Hct and platelets values.

Table (1): Red blood cells count (RBC's, 10⁶/µl), white blood cells count (WBC's, 10³/µl), platelets (10³/µl), hemoglobin,( Hb, g/dl) and hematocrit (Hct %) for control and different experimental groups:

<table>
<thead>
<tr>
<th>Rats group</th>
<th>RBC's (10⁶/µl)</th>
<th>WBC's (10³/µl)</th>
<th>Platelets (10³/µl)</th>
<th>Hb (g/dl)</th>
<th>Hct (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (G1)</td>
<td>5.15±0.31 ab</td>
<td>8.04±1.80 ab</td>
<td>560.00±85.27 a</td>
<td>9.00±0.44 a</td>
<td>28.36±1.39 a</td>
</tr>
<tr>
<td>LCPF (G2)</td>
<td>3.26±0.46</td>
<td>5.82±2.78 ad</td>
<td>281.60±40.41 b</td>
<td>5.64±0.76</td>
<td>18.46±2.68</td>
</tr>
<tr>
<td>HCPF (G3)</td>
<td>2.23±0.64</td>
<td>3.92±1.56 d</td>
<td>228.60±10.44 b</td>
<td>4.04±0.86</td>
<td>13.58±3.1</td>
</tr>
<tr>
<td>LCPF+Avemar (G4)</td>
<td>4.79±0.97 af</td>
<td>9.10±3.23 be</td>
<td>498.40±160.39 ad</td>
<td>8.58±1.75 af</td>
<td>27.40±5.54 a</td>
</tr>
<tr>
<td>HCPF+Avemar (G5)</td>
<td>4.22±0.66 f</td>
<td>8.38±3.83 be</td>
<td>484.20±118.39 ad</td>
<td>7.58±1.31 af</td>
<td>24.40±3.76 a</td>
</tr>
</tbody>
</table>

Values are expressed as means ± S.D, n=8.

LCPF: Low dose of CPF.

Significantly difference at P≤ 0.05

2. The effects of different experimental tested diets on mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC):

From the results in table (2) the indices levels of MCV and MCHC, show significant difference in case of rats fed on low and high doses of CPF and the percent of change from control group levels was 3.16% and -3.65% for those fed on low dose of CPF but it was 12.82% and -5.92% for those fed on high dose of CPF.

There was a significant difference between the MCH of rats fed on low CPF and G4, while MCHC and MCV reveal no significant difference. The group fed on high CPF illustrates a significant difference in respect to G5 only for MCHC while MCH and MCV show insignificant difference.
Table (2): Mean corpuscular volume MCV (Fl) mean corpuscular hemoglobin MCH (pg) and mean corpuscular hemoglobin concentration MCHC (g/dl) in different experimental groups.

<table>
<thead>
<tr>
<th>Rats group</th>
<th>Parameters</th>
<th>MCV (FL)</th>
<th>MCH (Pg)</th>
<th>MCHC (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (G1)</td>
<td></td>
<td>55.08± 1.03 a</td>
<td>17.50± 0.52 ab</td>
<td>31.74± 0.46 a</td>
</tr>
<tr>
<td>LCPF (G2)</td>
<td></td>
<td>56.82± 4.44 a</td>
<td>17.34± 1.26 a</td>
<td>30.58± 0.34 bc</td>
</tr>
<tr>
<td>HCPF (G3)</td>
<td></td>
<td>62.14± 10.22 c</td>
<td>18.48± 2.44 b</td>
<td>29.86± 1.46 b</td>
</tr>
<tr>
<td>LCPF+Avemar (G4)</td>
<td></td>
<td>57.30±3.30 a</td>
<td>17.90± 0.83 b</td>
<td>31.34± 0.56 ac</td>
</tr>
<tr>
<td>HCPF+Avemar (G5)</td>
<td></td>
<td>57.90±2.53 ac</td>
<td>17.96± 0.87 b</td>
<td>31.00± 0.60 ac</td>
</tr>
</tbody>
</table>

Legends as mentioned in table (1).

3. The effects of different experimental tested diets on serum alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) and gamma glutamyl transferase (γGT) activities:

The results of table (3) revealed that CPF intake in untreated groups induced liver injury which is reflected by the significant increase in all hepatic serum enzyme activities than control and treated groups. There is a noticeable improvement in all studied enzyme activities by avemar treatment as compared with G2 and G3.

Table (3): Serum alanine amino transferase (ALT), aspartate amino transferase (AST) U/ml and alkaline phosphatase (ALP) IU/l and gamma glutamyl transferase (γGT) U/l activities for control and different experimental groups.

<table>
<thead>
<tr>
<th>Rats group</th>
<th>Parameters</th>
<th>ALT U/ml</th>
<th>AST U/ml</th>
<th>ALP IU/l</th>
<th>γGT U/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (G1)</td>
<td></td>
<td>24.58± 4.81</td>
<td>36.93± 3.14</td>
<td>93.51± 15.32</td>
<td>2.64± 0.27</td>
</tr>
<tr>
<td>LCPF (G2)</td>
<td></td>
<td>50.75± 5.26</td>
<td>63.36± 4.57</td>
<td>211.16± 25.14</td>
<td>4.58± 0.51</td>
</tr>
<tr>
<td>HCPF (G3)</td>
<td></td>
<td>63.23± 3.49</td>
<td>76.42± 5.90</td>
<td>242.91± 45.39</td>
<td>7.60± 0.39</td>
</tr>
<tr>
<td>LCPF+Avemar (G4)</td>
<td></td>
<td>28.62± 3.60</td>
<td>40.65± 5.35</td>
<td>116.83± 17.08</td>
<td>2.65± 0.61</td>
</tr>
<tr>
<td>HCPF+Avemar (G5)</td>
<td></td>
<td>38.10± 3.26</td>
<td>49.75± 4.21</td>
<td>128.61± 17.08</td>
<td>2.72± 0.52</td>
</tr>
</tbody>
</table>

Legends as mentioned in table (1).
4. The effects of different experimental tested diets on serum total proteins, Albumin, globulins level and A/G ratio:

The results of table (4) demonstrated that, the administration of CPF either at low or high dose significantly decreased the levels of total protein, albumin and globulins. The improvement in serum total proteins and albumin levels was found in groups that received low dose of CPF plus avemar diet (G4), then (G5) that administered high CPF plus avemar diet when compared to control. The improvement was happened in globulins levels in all treated rats. Concerning A/G ratio, the effect of avemar diet in reducing the impact of CPF especially at low doses was higher than that of the G5.

Table (4): serum total proteins, albumin, globulins g/dl and A/G ratio in different experimental groups:

<table>
<thead>
<tr>
<th>Rats group</th>
<th>Total proteins g/dl</th>
<th>Albumin g/dl</th>
<th>Globulins g/dl</th>
<th>A/G Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (G1)</td>
<td>7.47±0.28 a</td>
<td>4.95±0.26 a</td>
<td>2.51±0.16 a</td>
<td>1.98±0.19 a</td>
</tr>
<tr>
<td>LCPF (G2)</td>
<td>5.21±0.38 a</td>
<td>2.88±0.24</td>
<td>2.32±0.20 b</td>
<td>1.25±0.11</td>
</tr>
<tr>
<td>HCPF (G3)</td>
<td>4.14±0.27</td>
<td>1.96±0.22</td>
<td>2.18±0.30</td>
<td>0.92±0.18</td>
</tr>
<tr>
<td>LCPF+Avemar (G4)</td>
<td>6.65±0.48 a</td>
<td>4.23±0.34 a</td>
<td>2.42±0.44 a</td>
<td>1.80±0.36 a</td>
</tr>
<tr>
<td>HCPF+Avemar (G5)</td>
<td>6.30±0.37</td>
<td>3.84±0.19</td>
<td>2.47±0.24 d</td>
<td>1.56±0.14 a</td>
</tr>
</tbody>
</table>

- Legends as mentioned in table (1).

5. The effects of different experimental tested diets on serum creatinine and urea levels:

The results demonstrated that administration of CPF at low dose cause significantly increase in creatinine and urea levels and highly increased in case of administration of high dose of CPF. The results demonstrated that administration of avemar (G4) to diet plus low dose CPF caused a significant decrease in creatinine level by -38.99% when compared to G2. While administration of avemar with diet plus high dose CPF caused a decrease in creatinine level (G5) by -51.62% when compared to high dose only of CPF (G3). Urea levels were improved in groups fed on avemar when compared with untreated groups.
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Table (5): Serum creatinine and urea (mg/dl) for control and different experimental groups

<table>
<thead>
<tr>
<th>Rats group</th>
<th>Creatinine (mg/dl)</th>
<th>Urea (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (G1)</td>
<td>0.79±0.13</td>
<td>24.22±4.04</td>
</tr>
<tr>
<td>LCPF (G2)</td>
<td>1.59±0.18</td>
<td>40.54±4.10</td>
</tr>
<tr>
<td>HCPF (G3)</td>
<td>2.46±0.34</td>
<td>49.48±4.95</td>
</tr>
<tr>
<td>LCPF+Avemar (G4)</td>
<td>0.97±0.20</td>
<td>24.45±2.99</td>
</tr>
<tr>
<td>HCPF+Avemar (G5)</td>
<td>1.19±0.11</td>
<td>27.91±1.68</td>
</tr>
</tbody>
</table>

Legends as mentioned in table (1).

6. The effects of different experimental tested diets on serum malondialdehyde (MDA) level and erythrocyte superoxide dismutase (SOD), plasma catalase (CAT), glutathione –S-transfrase (GST) activities:

From the results in table (6), the level of MDA, SOD, catalase and GST were statistically significant differences in groups that received low and high dose of CPF alone when compared with control.

Table (6): Serum malondialdehyde (MDA) level (nmol/ml) and erythrocyte superoxide dismutase (SOD) (U/g Hb), plasma catalase (CAT) (U/ml) and glutathione –S-transfrase (GST) activities (U/l) in control and different experimental groups.

<table>
<thead>
<tr>
<th>Rats group</th>
<th>MDA nmole/ml</th>
<th>SOD (U/g Hb)</th>
<th>CAT (U/ml)</th>
<th>GST (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (G1)</td>
<td>1.33±0.33</td>
<td>249.69±16.10</td>
<td>53.20±4.05</td>
<td>13.37±1.56</td>
</tr>
<tr>
<td>LCPF (G2)</td>
<td>2.68±0.38</td>
<td>151.46±33.65</td>
<td>35.44±4.11</td>
<td>9.63±0.95</td>
</tr>
<tr>
<td>HCPF (G3)</td>
<td>3.48±0.53</td>
<td>147.31±16.82</td>
<td>24.91±3.54</td>
<td>6.27±0.84</td>
</tr>
<tr>
<td>LCPF+Avemar (G4)</td>
<td>1.44±0.17</td>
<td>235.85±58.48</td>
<td>44.69±5.55</td>
<td>13.09±1.70</td>
</tr>
<tr>
<td>HCPF+Avemar (G5)</td>
<td>1.77±0.20</td>
<td>224.28±64.13</td>
<td>41.81±5.76</td>
<td>11.63±1.61</td>
</tr>
</tbody>
</table>

Legends as mentioned in table (1).
7. The effects of different experimental tested diets on serum total lipids, total cholesterol, triacylglycerols:

The results presented in table (7) showed that the presence of CPF in diets either at low or high dose induce significant increase in the level of serum total lipids, total cholesterol and TG. From the results of treated groups, there are noticeable improvements in all levels exhibited in treated groups by avemar when compared with groups fed on CPF only.

**Table (7):** Serum total lipids, total cholesterol, triacylglycerols (TG) mg /dl in different experimental groups.

<table>
<thead>
<tr>
<th>Rats group Parameters</th>
<th>Total lipids (mg/dl)</th>
<th>Total Cholesterol (mg/dl)</th>
<th>Triacylglycerols (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (G1)</td>
<td>369.39± 20.69</td>
<td>99.10± 5.83</td>
<td>143.32± 19.20</td>
</tr>
<tr>
<td>LCPF (G2)</td>
<td>635.01± 38.07</td>
<td>130.05± 17.01</td>
<td>162.06± 24.64</td>
</tr>
<tr>
<td>HCPF (G3)</td>
<td>791.53± 48.97</td>
<td>159.45± 17.26</td>
<td>192.35± 18.36</td>
</tr>
<tr>
<td>LCPF+Avemar (G4)</td>
<td>394.03± 31.41</td>
<td>78.00±8.10</td>
<td>137.26±24.02</td>
</tr>
<tr>
<td>HCPF+Avemar (G5)</td>
<td>418.15± 27.55</td>
<td>79.88±16.97</td>
<td>139.69±26.17</td>
</tr>
</tbody>
</table>

Legends as mentioned in table (1).

8. The effects of different experimental tested diets on serum (HDL-C), (LDL-C), (VLDL-C) and LDL-C/HDL-C ratio:

From the results shown in table (8) it was clear that there were significant differences between G2, G3 and control by increasing the level of LDL-C, VLDL-C, and LDL/HDL ratio. Avemar supplementation reduced the serum levels of LDL-C, VLDL-C, and LDL-C/HDL-C ratio and increased serum level of HDL-C.

**Table (8):** Serum (HDL-C), (LDL-C), (VLDL-C), and LDL/HDL ratio in different experimental groups.

<table>
<thead>
<tr>
<th>Rats group Parameters</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
<th>LDL/HDL ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (G1)</td>
<td>62.01±6.85</td>
<td>52.65±12.44 ab</td>
<td>28.66±3.84 ab</td>
<td>0.85±0.17 ab</td>
</tr>
<tr>
<td>LCPF (G2)</td>
<td>40.36±4.46 b</td>
<td>89.29±19.47</td>
<td>32.41±4.93  a</td>
<td>2.25±0.62</td>
</tr>
<tr>
<td>HCPF (G3)</td>
<td>33.14±5.14 c</td>
<td>120.74±16.27 d</td>
<td>38.47±3.67</td>
<td>3.77±1.12 d</td>
</tr>
<tr>
<td>LCPF+Avemar (G4)</td>
<td>59.14±6.17 c</td>
<td>50.67±20.71 ab</td>
<td>27.45±4.80 ab</td>
<td>0.88±0.39 a</td>
</tr>
<tr>
<td>HCPF+Avemar (G5)</td>
<td>56.81±6.28 ab c</td>
<td>55.16±22.12 ab</td>
<td>27.99±5.23 bf</td>
<td>1.00±0.45 b</td>
</tr>
</tbody>
</table>

Legends as mentioned in table (1).
9. Microscopical examination:

Figures (1-5) are sections of liver of rat (stained with H & E, X=150) showing:

Fig. (1): control group (G1) normal histological structure

Fig. (2): (G2): focal necrosis infiltration with inflammatory

Fig. (3): (G3): congestion of the portal area and inflammatory infiltration

Fig. (4): (G4): few vacuoles in the hepatocytes with displacement of the nuclei and the most of the hepatocytes

Fig. (5): (G5): focal necrosis and few vacuoles in the hepatocytes
DISCUSSION

Chlorpyrifos (CPF), an organophosphorus insecticide is known to cause oxidative stress in different human and animal cells (Jett and Navoa, 2000). Chlorpyrifos is a lipophilic molecule which can easily pass through the cell membrane into the cytoplasm. Once inside the cell, CPF can generate a lot of damages. For these reasons, it is necessary to find solutions against this danger. Within this context, nature can provide us many substances that can attenuate this oxidative stress (Gupta, 2006).

This study demonstrated that, CPF caused decrease in RBC count, Hb and Hct levels, which might be due to the effect of pesticide on blood forming organs suggesting the anaemic condition of the treated animals. The anemia may be due to the inhibition of erythropoiesis and hemosynthesis and to an increase in the rate of erythrocytes destruction in hemopoietic organs, as well as the leucocytosis observed in present study indicates an immune system to protect the rats against infection that might have been caused by chemical and also secondary infections, which may be contracted after the weakening condition of the rats. Leucocytosis, which may be directly proportional to the severity of the causative stress condition, may be attributed to an increase in leukocyte mobilization. The present treatment normalized the levels of blood cell counts, and other indices. The protective effects of avemar are most likely due to its antioxidant potential.

The present results are in harmony with the results of Akhtar et al. (2009) who reported that, the Hb and Hct levels were significantly decreased in animals exposed to CPF at 3, 6 and 9 mg/kg/d doses. Also Janeway, (2005) explained the reduction in the number of lymphocytes may be due to decreased production or rapid removal from circulation and subsequent destruction. The reduction of lymphocytes is indicative of immunosuppressive effects of organophosphates which may require other studies to assess the levels of immunoglobulins. Moreover, the results obtained by Goel et al. (2006) confirmed that, decrease in leukocyte counts following intoxication with CPF could be attributed either to the slower rate of production of leukocytes or due to their inhibited release into the blood circulation. A significant decrease in the total bone marrow cell count was indicated. The results of Kazmi et al. (2003) showed the effect of sublethal dosage (120 mg/kg bw/day) of the organophosphate pesticide, CPF on the blood of adult male albino Sprague-Dawley rat under short-term conditions. CPF had no effect on hemoglobin. Red blood and white blood cell counts and packed cell volume showed a significant but transient decline. The mean corpuscular volume and mean corpuscular hemoglobin also increased transiently. Mean corpuscular hemoglobin concentrations showed persistent increase. It has been shown by Philipp et al. (2007) that avemar, a fermented wheat germ extract standardized to methoxy-substituted benzoquinones, possesses cancer-fighting characteristics. Taken orally, avemar can inhibit metastatic tumor also, avemar was demonstrated to induce apoptosis in pancreatic carcinoma cells, T and B lymphocytic tumor cell lines, leukemia, melanoma, breast cancer and gastric carcinoma cells in vitro.

Increase in serum ALT, AST activities as observed in groups treated with CPF may reflect damage of liver cells and cellular degeneration or destruction occurs
in this organ and the increase in the activity of ALP in serum activity might be due to the increased permeability of plasma membrane or cellular necrosis, and this showed the stress condition of the treated animals with CPF. Also, the results of the present study indicate that avermar significantly reduces the toxic effects of CPF by altered the hepatic enzyme activities and thus can be considered a potential protective agent in conditions of organophosphate poisoning. The organophosphorus insecticides induce an obvious increase in AST, ALT, ALP activities, this fact is a conventional indicator of liver injury that reported by Rao (2006). When the liver cell membrane is damaged, varieties of enzymes normally located in the cytosol are released into the blood stream. Elevation of AST and ALT activities indicates the utilization of amino acids for the oxidation or for gluconeogenesis and is used to determine liver damage (Etim et al., 2006). Also, the elevation in ALP activity suggests an increase in lysosomal mobilization and cell necrosis due to pesticide toxicity (Kalender et al., 2005). Normally, the reduction of albumin level indicates a liver disease. This reduction could be attributed to changes in the protein and free amino acid metabolism and their synthesis in the liver (Ncibi et al., 2008). In the same field, Li et al. (2007) suggested that albumin could be used as a biomarker of CPF toxicity. Moreover, Heimbach et al. (2007) demonstrated that the clinical chemistry values remained within normal ranges, with the exception that control males had statistically significantly higher AST (124.70±25.56 U/L versus 83.70±16.04 U/L) and ALT (65.23±7.57 U/L versus 54.73±6.07 U/L) activities compared to treated males after 28 days of treatment by avermar 2000 mg/kg bw/day.

In the present study, CPF treatment caused significant increase in the serum creatinine and urea levels of rats and changes in relative kidney weights. The creatinine excretion is dependent almost on the process of glomerular filtration, although tubular secretion contributes slightly. However, the slight and significant rise in the serum creatinine and urea levels of rats may be due to the impairment of the glomerular function and tubular damage in the kidney. The results indicated that co-administration of avermar to CPF intoxicated rats reverted most of these altered biochemical parameter levels to be within normal limits and improved liver and kidney dysfunction. Urea and creatinine are waste products of protein metabolism that need to be excreted by the kidney, therefore a marked increase in serum urea and creatinine, as noticed in the study confirmed an indication of functional kidney damage. Urea level can be increased by many other factors such as dehydration, antidiuretic drugs and diet, while creatinine is more specific to the kidney function of Garba et al. (2007). Since kidney damage is the only significant factor that increases the serum creatinine level (Nwanjo et al., 2005). Therefore, significant increases in urea by 70.5± 1.90 and 83.5± 1.78 mg/dl for low and high dose of CPE, respectively and creatinine levels was 2.33± 0.10 and 3.16± 0.13 mg/dl for treatment with low and high dose of CPE, respectively. In this study, a classical sign that the kidney was adversely affected by CPF administration. Moreover, a reduction in the glomerular filtration rate was observed through the increased creatinine and urea in plasma along with decreased plasma albumin. The serum albumin concentration may be directly altered, due to increased loss of albumin through damaged glomeruli in case
of renal failure detected by Venkatesan et al. (2000).

In the present investigation CPF has been postulated to have multiple effects on the target cells including generation of reactive oxygen species and induction of intracellular oxidative stress, thereby, disrupting normal cellular development and differentiation. It is interesting to find that the avermar supplementation resulted to high GST and SOD activities as well as of control group, which may indicate that the treated nutrients helped glutathione synthesis, in addition to its role as an essential component of SOD that is vital to cellular antioxidant defence. It has also been reported to interact with cell membranes to stabilize them against various damaging effects, including those due to oxidative injuries (Gutteridge and Halliwell 2000). Goel et al. (2005) reported that highly reactive oxygen metabolites, especially hydroxyl radicals, act on unsaturated fatty acids of phospholipid components of membranes to produce malondialdehyde, a lipid peroxidation product. This is agreement with our results since CPF have been reported to induce oxidative stress, as shown by enhanced MDA production. Juskiewicz et al. (2002) demonstrated significantly lower content of malondialdehyde suggested that the addition of flavonoid extract increased the antioxidative potential of serum. While Boros et al. (2005) demonstrated that avermar contains compounds such as benzoquinones and other plant flavonoids, important agents in controlling oxidative stress and cell damage.

Organophosphorus (OP) insecticides generally increase the total cholesterol and total lipid levels (Lasram et al., 2009) and indeed, in the present study, CPF elevated the total cholesterol levels of the rats. This increase in serum cholesterol can be attributed to the effect of pesticides on the permeability of the liver cell membrane (Yousef et al., 2006). In addition, the increase in serum total cholesterol levels may be due to the blockage of the liver bile ducts, which reduces or stops cholesterol secretion into the duodenum (Ogutcu et al., 2008). As observed in the present study, avermar, grape seed oil and wheat germ oil treatment can decrease serum total cholesterol level and can improve serum lipid profile to a significant extent. These results show that treatment extracts exert considerable antioxidant potency in vivo as well, and protect cellular structures against peroxidation.

Gutteridge and Halliwell (2000) showed that CPF treatment to normal rats led to marked increase in the hepatic lipid peroxidation (LPO). LPO is the process of oxidative degradation of polyunsaturated fatty acids and its occurrence in biological membranes caused impaired membrane function, structural integrity showed by decrease in membrane fluidity and inactivation of a several membrane bound enzymes, so CPF treatment result in peroxidation of polyunsaturated fatty acids, leading to the degradation of phospholipids and ultimately result in cellular deterioration. The flavonoids and phenolic acids Coenzyme Q10 (have very similar characteristics to vitamins and are antioxidant compounds) and alpha-tocopherol found in avermar decreased intracellular triacylglycerols (Hsu and Yen, 2007). Also avermar contains compounds such as benzoquinones and other plant flavonoids, which are important agents in controlling oxidative stress and cell damage (Iyer and Brown, 2009). Moreover, Pepe et al. (2007) reported that components of avermar such as the benzoquinones may also be cardioprotective. Benzoquinones have very
similar characteristics to vitamins and are antioxidant compounds. Coenzyme Q10 (ubiquinone) is a naturally occurring benzoquinone, which may prevent cellular damage during myocardial ischemia and reperfusion by its roles in oxidative phosphorylation and membrane stabilization. Treatment by α-tocopherol decreased glycated hemoglobin A1c (HbA1c) and pancreatic lipid peroxidation in diabetic rats. Coenzyme Q10 has also been used in oral form to treat various cardiovascular disorders including hypertension and congestive heart failure (Salamah 2010).

Concerning the hepatic histoarchitecture of the CPF-treated animals there was an increased vacuolization of hepatocytes and focal necrosis in comparison to untreated normal controls. The congestion of the portal area, inflammatory infiltration increased in these animals. These observations indicated the marked changes in the overall histoarchitecture of liver in response to CPF, which could be due to its toxic effects. Primarily by the generation of reactive oxygen species causing damage to the various membranous components of the cell. The necrotic conditions observed in liver of CPF treated animals are in correlation with the observed biochemical changes, wherein an increased level of lipid peroxidation was noticed. The supplementation of avemar is recommended as a concomitant supplementation to the routine therapy for the protection against severe tissue damage induced by the organophosphorus. Focal necrosis of liver as observed in this study following chloryrifos treatment has been reported earlier by Jee et al. (2005) opined that vascular formation is a cellular defense mechanism against injurious substances to cells; these substances were segregated in vacuoles and thus were prevented from interfering with cellular metabolism. It has also been suggested that cytoplasmic vacuolation is mainly a consequence of disturbances in lipid inclusions and fat metabolism occurring during pathological disturbances.

REFERENCES


Biochemical role of fermented wheat germ on liver and kidney functions alteration induced by chlorpyrifos in rats


الدور البيوكيميائي لجنيه القمح المتخمر على تغير وظائف الكبد والكلى المحدث بالكلوروبيريفوز في الجرذان

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الكلوروبيريفوز هو مبيد حشرى عضوى فوسفاتي يستخدم على نطاق واسع للعديد من التطبيقات الزراعية والصحية العامة. إن الغرض من هذه الدراسة تقييم الدور البيوكيميائي لجنيه القمح المتخمر (الافيمار) على وظائف الكبد والكلى والجهد التناسقي المحدث بالكلوروبيريفوز في الجرذان، وكذلك تكرير صوره الدم والفحص النسيجي للبد. اضيف الكلوروبيريفوز للوجبات المختبرة جرعتين، المنخفضة والمرتفعة (25 و 50 مجم/كمج وجبة على التوالي). بينما أضيف الافيمار بمقدار 3 جم/كمج وجبة. أظهرت النتائج انخفاض مرئي في الكلى لكرات الدم الحمراء والبيضاء ومؤشرات كرات الدم الحمراء وتركيز الهيموجلوبين ومستوى الليماتوركبي في الجرذان التي تناولت كل من تركيز الكلوروبيريفوز، وكذلك تأثير وظائف الكبد مع زيادة نشاط الانزيمات ALT, AST, ALP, γGT. بينما انخفض تركيز البروتينات الكلي والألبومين والجلوبولين في المجموعات التي تناولت الافيمار، بالإضافة إلى انخفاض الكرياتينين والبروتين في زيادة اكشده الدهون ومكونات دهن الدم، بينما انخفض نشاط الانزيمات SOD, CAT, GST معنويًا. و لذلك فإن التدعيم بالافيمار يعمل على تحسين معنوي في كل القياسات التي اجريت مقارنه بمتاليتها بالمجموعات التي تناولت الكلوروبيريفوز منفردا.