Full Length Research Paper

Effect of organophosphate malathion on tissues of quail (Coturnix coturnix)

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The aim of the present study to investigate effects of the organophosphates insecticides malathion on hematological and histopathological changes in liver and illume tissues of quail (Coturnix coturnix). Quail were treated orally with 1/4 LD50 (25mg /kg B.W.) of malathion for 28 days. Malathion decreased the body weight significantly at the end of experimental. There were statistically significantly decreased in hemoglobin content, and increased the number of leucocytes in malathion treated- quail as compared to control. There are changes in the liver tissues as indicated by cytoplasmic vaculation, congestion of liver portal vein, infiltration inflammatory cells in many parts of the hepatic lobe and increasing the number of Kupffer cell. The histopathological changes in the illume tissues showed increased in the size and number of goblet cells and cytoplasmic vaculation on columnale epithelial cell. We can conclude that the malathion induced toxicity in some blood parameters and caused histopathological changes in the liver and illume of quail.

Keywords: Organophosphates insecticides, Malathion, Histopathological changes, Coturnix coturnix.

INTRODUCTION

Organophosphate insecticides (OPI) are globally used in the control of insects around the home and in agriculture practice (Donaldson, et al., 2002). There have been increasing concerns about the effects of various OPI in humans and experimental animals. These include cholinergic and noncholinergic biological disturbances (Gordon and Mack, 2003). Exposure to these insecticides can involve a large segment of the population including, agriculture workers and their families, those living in proximity to farms, and the general population who may be exposed through home application of pesticides or via residues on food (Bradman et al., 2003). OPI like malathion (S-(1,2-BIS-(ethoxy carbonyl)-ethyl)-O,O-dimethyle phosphorodithioate) has been extensively used wildly for the control of agriculture and household pets. Some reports have been published with respect to the chronic malathion toxicity on some histological parameters of quail (Kalender et al., 2005). The toxicity of malathion has been studied before by Dutta et al. (2004), exposure to malathion at a dose (50 mg /kg) decreased the weight of the rabbits that treated for 28 days. Koc (2008) when he introduced rats to malathion at dose (1mg/kg) for 15 days found that there were increased in the number of lymphocytes. The toxicity of malathion is not only on the body weight and blood parameters but also on Liver and instant tissues. Caglar and Kolankaya (2007) reported that the rats exposed to malathion at a dose of 56 or 560 mg /kg had the liver balloon. Ozlemas and Akay (1995) noticed on lizard exposed to the different concentrations of malathione (1, 2, 3 mg / g) for 2 weeks, the number of goblet cells increased and they found accumulation of mucus in the instant cytoplasm.

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There is no data available on the effect of malathion on hematological parameters of quail as well as on the histological changes of ileum. Therefore, the present work is initiated to study the effects of this insecticide on hematological parameters, liver and ileum tissues of quail.

MATERIALS AND METHODS

Birds

Male quails (*Coturnix coturnix*) were brought from commercial marketing in Jeddah K.S.A. They weight (197.6g ± 1.8g), and maintained in a laboratory environment for two weeks at a temperature (24 ± 2 °C) with food, and water ad libium for adaptation on the new environmental.

Chemical

Malathion 57% was applied as commercial emulsifiable concentrate formulation containing 57 % active ingredient. It was diluted in deionized water for the final concentration.

Experimental design

Birds were divided into two main groups: Groups A is control group, in which the birds were (n= 10) received saline (25mg /kg B.w.) orally for 28 days. Groups B is treated-group, in which the birds were (n= 10) received LD50 (25mg /kg B.w.) orally for 28 days according to Al Qarawi and Adam (2003). After 14 days and at the end of treatment period, body weight, blood samples, liver and intestine were taken for hematological and histopathological analysis.

Body weight and hematological parameters determination

All animal were weighed by automatic balance (AND GX-600, japans) at 0, 14 and 28 days and compared with control groups. Blood sample were collected from all birds from the wing vein according to the method of Das et al. (2004). Blood sample were transferred to test tube containing EDTA for hematological parameters [red blood cell count (RBC), hemoglobin (Hb) content, white blood cell count (WBC) and blood clotting time were determined] using cell counter at different time (0 , 14 and 28 days). Blood smearing was used for studying the general shape of blood cell by using the method of Karabay and Oguz (2005) at different times.

Histological study

The liver and ileum tissues were dissected and the tissue sample were fixed in 10 % formalin solution for 24 hrs, passed in series of graded ethanol and embedded in paraffin. Paraffin section were cut at 5µm thickness and stained with hematoxiline and eosin for light microscope examination (Salih,1999). The section were examined and photographic on an Olympus light microscope (Olympus B×51, Tokyo, japan) with an attached camera (Olympus C-5050, Olympus Optical Co. Ltd., Japan).

Statistical analysis

Statistical analysis was based on comparing the value between the malathion groups with control groups. The result was expressed as mean ± SD of 5 birds / group. The statistical significant of the date has been determined using one way Analysis of Variance (ANOVA – LSD) using SPSS statistical software package version 13. The level of significant was taken below P<0.05.

RESULT

Evaluation of body weight

In the beginning of this study, the mean body weight of quail was (197.6g ± 1.8g). Significant different were observed in body weight between malathion-treated
group and the control birds (Table 1). A significant decline \((p<0.05)\) of the mean body weight was observed after administration malathion for 14 days (Table 1). The food intake by quails was decrease significantly. By increase the time of treated to 28 days, most of malathion–treated quail was decreased in their body weight significantly as compared to 14 days-treatment birds.

**Change in hematological parameters**

Hematological change were shown in Table (2). A significant decrease was observed in Hb at the end of experimental in malathion- treated group compared to control group \((p< 0.05)\).

Blood clotting time change were shown in Table (2). A significant decrease was observed in blood clotting time after 14 days treatment. The blood clotting time was 11.2 min for malathion treated group as compared to 8.6 min for control group \((p<0.05)\). At the end of experimental in malathion treated group has the high significant of blood clotting time as compared to control group \((p< 0.01)\).

**Table 2.** Hemoglobin content and blood clotting time of quail \((Coturnix coturnix)\) that treated with malathion \((25 \text{ mg} / \text{kg b.w.})\).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time (days)</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
<td>14</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
</tr>
<tr>
<td>Hb (g/L blood)</td>
<td>13.48 ± 1.21</td>
<td>14.56 ± 0.55</td>
<td>13.96 ± 1.29</td>
<td>12.8 ± 1.09</td>
<td>13.4 ± 0.91</td>
</tr>
<tr>
<td>Blood clotting</td>
<td>9.1 ± 0.54</td>
<td>8.6 ± 0.54</td>
<td>9.6 ± 0.54</td>
<td>11.2 ± 0.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.6 ± 0.54</td>
</tr>
</tbody>
</table>

Values represent as mean ± SD \((n=5, \text{in each groups})\). The birds had been treated with malathion \((25 \text{ mg} / \text{kg b.w.})\) orally. <sup>a</sup> Significant different as compared to control \(P< 0.05\). <sup>b</sup> Significant different as compared to 14-days treated-group, \(P<0.01\).

**Figure 1.** Photomicrograph of Gemesa staining smear of blood \((1000X)\). A, blood from control quail \((R: \text{RWCs, 1000×})\); B, blood from quail that treated with 25 mg/kg B.W. of malathion for 14 days where WBCs (W) increased, cytoplasmic vaculation (black arrow); C; blood from quail 25 mg/kg B.W. malathion–treated group for 21 days where there are chromatin granulars (Black arrow) and reticular cell (RC). D, blood from quail that treated with 25 mg/kg B.W. malathion for 28 days cell membrane break down (Black arrow).

Red oval blood cells with central nuclei and the presence
of the spread of simple white blood cells were observed in control birds (Figure 1A). After 14 days of treatment, smear of blood malathion – treated groups, showed that changes in blood cell indicated by increasing the number of WBCs, irregular circular shape and cytoplasmic vaculation on RBCs (Figure 1B). After 21 days of treatment quail there were differences in distribution of chromatin granules inside the nucleus of RBCs and increase secretion the reticular cell (Figure 1C). The most apparent change after 28 days of treatment by malathion have been observed many changes in RBCs that indicated by break down cell membranes (Figure 1D).

Histopathological finding

Results of histopathological examination of liver sample obtained from the control group within normal limits (Figure 2A). Control liver tissue showed normal cellular architecture with distinct hepatic cells, sinusoids spaces. This sinusoid have irregular boundaries composed of only a signal layer of fenestrated endothelial cells and large irregular phagocytes cells which are known as kuffer cells and central vein. Similarly, the malathion group showed several signs of toxicity as necrotic cells.

Examination of liver section for treated quail after 14 days observed a significant increase in the number of Kuffer cell (Figure 2B) and the start of the cytoplasmic vaculation in cytoplasm of liver cells (Figure 2 C). After 21 days, congestion blood on portal and central vein (Figure 2E), increase the size of the cytoplasmic vaculation and liver cell transformation to a balloon were observed (Figure 2 F). After 28 days, at treated cell membrane was break down, cells necroses and increase inflammatory infiltration of cell (Figure 2 J).

The toxicity of malathion not only reach the liver tissues, but also intestines tissues. The mucous layer, one of the illumie tissue layers of interior, which appear in the form of extended fingers called (Villi) (Fig 3A). After 14 days of treatment the quail with malathion, the numbers and size og goblet cells increased as compared to control group (Figure 3 B). After 21 days, there was cytoplasmic vaculation and separation of the columnar epithelial cells from each other (Figure 3 C, 3 D). By the end of experimental, it is difficult to distinguish the different part of intestine (Figure 3 E).

DISCUSSION

Use the pesticide malathion on a commercial scale and widely as a pesticide for more than 58 years as used in the fight against scale insects and some types of flies. It had also been used to fight many diseases by eliminating
the insect vectors of disease such as malaria the mosquito-borne, and was used to control animal parasites. (Mason and Epple, 1999). OPI cause reduction of body wight in experimental animals (Saleh, 1999). In the present study, malathion was given ¼ LD50 does (25 mg / kg B.W.) and no death was observed during experiments. A decrease in body wight was observed after 14 days of malathion treated. That could be due to the reduced food intake. The same observation has been reported in rabbit exposure to malathion at a dose 50 mg/kg for 28 days (Dutta et al., 2004). In the present study, we observed changes of some hematological parameters in quail, the Hb content was decreased leading to anemia after 14 days of treatment (Rastogi et al., 2008; Rezg et al., 2007, Chakrarty and Banerje, 1988). The reduction in Hb cotenants could be attributed to internal hemorrhage or increase in the quail of erythrocytes destruction in hemopoietic organs. A similar trend has been observed in rabbit treated with dizanon (Yehia et al., 2007), they found that Hb cotenants decreased with dizanon. The some observation was reported by Rezg et al. (2007) they found that increase in the blood clotting time. There was increase blood clotting in this reference guide that there is damage to the liver cells and followed by an imbalance in the liver and the production of blood proteins help the blood clotting process and is a clear indication of the damage made in the liver tissue. Ahmad et al. (2000) noticed that the malathion caused damage to various physiological blood cells and increasing the number of WBCs. Moreover, the the number of lymphocytes were increased in mice treated with 1 mg/kg of malathion for 15 days (El-Shenawy et al., 2010)

Treating animals with malathion induced many histopathological changes in the liver. The most prevalent symptoms of hepatic tissue impairment were destruction of liver architecture, cytoplasmic vaculation of the hepatocytes and remarkable abundance of leucocytes infiltration (Yehia et al., 2007). These alteration seemed to follow almost the same pattern as that previously explained by many investigators under the effect of different insecticides. Studies on the effect of different insecticides on mammalian liver have been repeated El-Shenawy et al. (2010) including necrosis, blood vessels congestion and leucocytes infiltration in the liver of experimental animals treated with pyrethroids.

The main pathological manifestations observed significantly in the mucous layer of the intestine, where they formed a group of cytoplasmic gaps within the epithelial lining of the vertical villi leading to further push

Figure 3. Photomicrograph of HE staining section of intestine (1000X). A, section of illume from control quail; B, section of illume from quail 25 mg /kg b.w. malathion treatment group 14 days, increase number of goblet cell (Black arrow); C, section of illume from quail 25 mg /kg b.w. malathion treatment group 21 days, broken for intercellular and the upper of columnar epithelium cell (Black arrow); D, section of illume from quail 25 mg /kg b.w. malathion treatment group 28 days, full broken for illume cells (Black arrow).
its expansion nuclei on the cell membrane and increase the pressure on the membrane led to the torn and exit the nucleus (Rastogi et al., 2008). Moreover, there that was noted for the cells filled with mucus and increase their number as compared to the control birds in a case adaptive to reduce the absorption of the pesticide and put it out and this result agrees with Ozlemas and Akay (1995) on lizards exposed to depended (1, 2, 3 mg / kg) of malathion for two weeks. (Rastogi et al., 2008; Rezg et al., 2007, Chakrarty and Banerje, 1988), they noticed an increase in the number of cells and accumulation of mucus in the intestine cytoplasm. Increasing the time of exposure to malathion lead to increase the size of epithelium cells.

This could be explained to changes in Na\(^+\),K\(^+\) pump and impaired the entry of these elements as a result of sodium entry followed by the entry of water into gaps within the cell component (Chowdhury, et al., 1980) when he offered to male rats at a dose of Malathion (1 mg / kg) noted the disruption to pump sodium and potassium.

In calculation, we could concluded that effect of malathion at dose of 25 mg/kg B.W. on male quail adult caused changes in some hematological parameters and histopathological changes on liver and illume.

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