Effects of Exposure to Diazinon on the Lung and Small Intestine of Guinea Pig, Histological and Some Histochemical Changes

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ABSTRACT

The present study aimed to evaluate the histological changes and adverse effects on the total protein and general carbohydrates in the lung and small intestine of guinea pig "Cavia porcellus" induced by the exposure to sub lethal doses of the organophosphate insecticide diazinon. Primarily, diazinon insecticide oral LD$_{50}$ was investigated after administrating orally different doses in guinea pig and was determined as 213 mg/Kg bw., the animals exposed to sub lethal doses of this insecticide revealed histological changes in both lung and intestine in a dose dependent manner. Also, significant decrease in the total protein and general carbohydrates in the lung and intestine were mostly observed, especially with the higher doses of the insecticide in comparison with control group.

Key words: Diazinon, Guinea pig, Lung, Intestine

INTRODUCTION

Considerable toxicity data of chemicals have been produced in the past few years. The environmental contamination with pesticides is a problem of the regional as well as world-wide importance (EPA, 1999). The toxic chemicals have been found in a variety of environmental samples including water, air and house dust, and their presence has also been noted in the tissues of non-occupationally exposed people, particularly in the adipose tissue, blood and urine (Wagner et al. 1991).

The organophosphate insecticide diazinon has agricultural, commercial, and household uses, but the household uses predominate. The crops using the most diazinon are almonds, berries, pecans, and nectarines (EPA, 1999). As most organophosphates, diazinon attaches to acetyl cholinesterase and prevent it from destroying acetylcholine causing over stimulation of the nerves (Ware, 2000). Also, diazinon and other organophosphates inhibitor numerous enzymes with molecular structure that are similar to AChE (Reigart and Roberts, 1999).

The prolonged exposure to the most commonly used agricultural pesticides increase the risk of the lung lesions, as well as cancer, in the farmers and commercial pesticide users. However, this increased risk was only significant for the prolonged exposure to diazinon, dieldrin, metalochlor and pendimethalin (Dinham, 2005). Also, the histological changes in the alimentary canal of experimental animals due to pesticides exposure have been documented (Poet et al., 2003)

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Several studies have been made on the effect of the exposure of the experimental animals, including guinea pig, to the pesticides (Purdey, 1994; Reigart and Roberts, 1999; Dede and Chike, 2000 and Dede and Dogara, 2004). However, limited information is available on the subject of detection of the exposure effects to the minor concentrations of diazinon insecticide on the internal organs of guinea pig, *Cavia porcellus*. The aim of this work was to study the histological changes induced by the sub-lethal doses (1/20, 1/10 and 1/5 LD$_{50}$) of diazinon insecticide on the lung and small intestine of guinea pig, *Cavia porcellus*. In addition, the changes in the total protein and carbohydrates were investigated in these tissues histochemically.

**MATERIALS AND METHODS**

**Animals**
A total of 37 male animals belonging to the common species of guinea pig *Cavia porcellus* were used in this study. Live and healthy specimens of these animals were collected from the markets at Cairo Province, Egypt and maintained in special cages in the animal house at room temperature for two week for acclimatization before the beginning of the experiment. The average body weight of these animals was 540±45gm. The animals were fed *ad libitum* daily with green meal (alfalfa) and supplied with water.

**Insecticide used**
The insecticide used in this study, diazinon, was obtained from The Central Pesticides Laboratory (CPL), Ministry of Agriculture, Egypt. They were samples of that used in the fields (82% purity). The molecular formula is: C12-H21-N2-03-P-S and the structure formula is: (C2H5O)2-P(=S)-O-C4HN2-(CH3)-CH(CH3)2 (C4HN2 pyrimidine ring).

**Determination of LD$_{50}$**
A pilot study was carried out with 25 male guinea pigs (divided into five groups) to determine the minimum dose of diazinon that caused 100% death of the animals. The animals were observed for five days and a dead animal was removed immediately (an animal was considered dead after the stop of movements, loss of body reflexes). The number of dead animals was recorded and the mortality rate was calculated as a percentages. LD$_{50}$ was later calculated according to the equation by Behren and Karber (1953).

**Induction of the insecticide**
Acclimatized animals were divided in four groups, three animals/ group and kept in separated cages. The first group was used as control, while the other three groups (TI, TII and TIII) received 1/20, 1/10 and 1/5 of LD$_{50}$ of the insecticide, respectively, orally through gastric tube. All the cages had the same conditions mentioned at the period of the acclimatization. The animals in the treated cages as well as in control cage were observed for at least two hours after the insecticide was introduced and then were observed at time intervals. All the treated groups received eleven doses through three weeks; the dosage was day after day. After three weeks, the specimens were taken from each treated and control cages and anesthetized with ether and immediately dissected.

**Histological and Histochemical Investigations**
For the histological investigations, the parts of lung and small intestine from the control and the treated animals were fixed in neutral formalin. After the fixation period, the tissues were washed in the tap-water, dehydrated through a graded series of ethyl alcohol, cleared in xylene, embedded in the paraffin wax or parablast, sectioned at a thickness 5-7 microns, mounted on the glass slides and stained with haematoxylin and eosin for general morphological studies. The various slides based on the dose ranges were studied and the tissues were compared with the control, 0.9% saline injected guinea pigs. For the histochemical investigation, polysaccharide materials were illustrated following the application of periodic acid Schiff’s (PAS) technique (Huomason, 1979). For visualization of the total proteins, the mercury bromphenol blue method of Mazia et al. (1953) was applied. The study of total protein and polysaccharide materials was done using computer image analyzing system (Leica Model). The estimation of the optical density of thirty cells in each group was made. The data obtained were statistically analyzed according to Sendecor (1987).
RESULTS

The LD$_{50}$ value of diazinon in the male guinea pigs using the oral route of administration was 210 mg/kg body weight. Table 1 illustrated the symptoms that appeared in LD$_{50}$ experiment, these signs showed firstly with the calmness followed by irritability, tremor, motor incoordination, staggering and convulsion denoting neurological effects. Also, the manifestation of laboured breathing and ataxia were prominent in most groups. The oral dose of diazinon equal 270 mg/kg resulted in 100% mortality.

Histological effects

Lung

Normal structures of the lung alveoli obtained from the control guinea pig are depicted in Fig. (1 A). The normal alveoli were lined with the normal squamous cell (type I pneumocytes) and secretory cells (type II pneumocytes) with some macrophages. Inspected lung sections obtained from T1 (1/20 LD$_{50}$) revealed haemorrhage in different parts and increase of the dust cell within the interalveolar septa (Fig. 1 B). The lung of guinea pig treated with 1/10 LD$_{50}$ of diazinon exhibited congestion in the blood vessels and infiltration of the lymphocytes in the tissues (Fig. 1 C). Also, haemorrhage and increases in both dust cells and macrophages around and within the alveoli were observed. Some alveoli were obliterated due to proliferation and hypertrophy of the alveolar cells (Fig. 1 D). The high dose of diazinon (1/5 LD$_{50}$) induced severe lesions in the lung of treated animals. These lesions were manifested in prevailing haemorrhage, infiltration of macrophages and mononuclear cells, oedematous tissue, pyknotic nuclei and necrotic cells (Figs. 1 E and F).

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Dose (mg/kg b w)</th>
<th>Mortality rate</th>
<th>LD$_{50}$</th>
<th>Signs and symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>2</td>
<td>180</td>
<td>20%</td>
<td>213 mg/Kg b w</td>
<td>- Laboured breathing, tremor and irritability (4). Death (1)</td>
</tr>
<tr>
<td>3</td>
<td>210</td>
<td>40%</td>
<td></td>
<td>- Irritability and laboured breathing (3). Tremor (3). Staggering and convulsion (3). Death (2)</td>
</tr>
<tr>
<td>4</td>
<td>240</td>
<td>80%</td>
<td></td>
<td>- Irritability and laboured breathing (5). Staggering and convulsion (4). Death (4).</td>
</tr>
<tr>
<td>5</td>
<td>270</td>
<td>100%</td>
<td></td>
<td>- Laboured breathing (5), irritability (5), tremor (5), staggering (4) and convulsion (5). Death (5).</td>
</tr>
</tbody>
</table>

Table 1 - The mortality rate; LD$_{50}$ and signs and symptoms of diazinon insecticide toxicosis on *Cavia porcellus* (data collected 5 days after exposure).
**Intestine**

The normal villi of the small intestine (jejunum) of guinea pig are shown in Fig. (2 A). Inspected intestinal sections obtained from the animals treated with 1/20 LD_{50} of diazinon revealed slight changes. These changes were represented mainly in the infiltration and hypertrophy of the lymphocytes (Fig. 2 B). The treatment with 1/10 LD_{50} of the insecticide resulted in haemorrhage in the submucosa, aggregation of lymphocytes and erosion in the lining epithelium of the small intestine of the treated animals specially in the duodenal area (Figs. 2 C and D). Also, the normal pattern of villi was mostly ill defined.

Sever histological effects were observed in the small intestine post treatment with the high dose (1/5 LD_{50}). The erosions were perceptive in different parts of the intestine and the cells of the lining epithelium and crypts exhibited pyknotic nuclei. In addition, necrotic cells and degenerated areas were greatly encountered (Figs. 2 E and F).

**Histochemical effect**

**Total proteins**

The total proteins were indicated as blue colour with bromphenol blue stain, the average of total proteins in the lung of control animals recorded 1.576 (Fig. 3 A and Table 2). This average

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**Figure 1** - A - T. S. of control guinea pig lung showing normal alveoli; squamous cells (thin arrows), secretory cells (thick arrows) and macrophages (M.). (H&E stain).

B - T. S. of the lung of TI showing haemorrhage (thick arrows) and increase of dust cells (thin arrows) between the alveoli. (H&E stain).

C - T. S. of the lung of TII showing congested blood vessel (CBV) and infiltration of lymphocytes (thin arrows). (H&E stain).

D - T. S. of the lung of TII showing haemorrhage (H), and increases of lymphocytes (L) and dust cells (D). (H&E stain).

E - T. S. of the lung of TIII showing oedema (O). (H&E stain).

F - T. S. of the lung of TIII showing increase of macrophages (thin arrows), pyknotic nuclei (short arrows) and necrotic cells (thick arrows). (H&E stain).
recorded 1.408, 1.0 and 0.805 in TI, TII and TIII respectively. This decrease was significant with the high dose only; the percentages of decrease recorded 10.66, 36.55 and 48.92% in TI, TII (Fig. 3 B) and GIII (Fig. 3 C), respectively. The average of the total proteins in the intestine of the control animals was 2.066 (Fig. 4, A and Table 2). This exhibited significant decreases in all insecticide treated groups. The percentage of changes recorded -31.12, 145.15 and -65.97% in TI, TII (Fig. 4 B) and TIII (Fig. 4 C), respectively. The average of the total proteins in the intestine of the control animals was 2.066 (Fig. 4, A and Table 2). This exhibited significant decreases in all insecticide treated groups. The percentage of changes recorded -31.12, 145.15 and -65.97% in TI, TII (Fig. 4 B) and TIII (Fig. 4 C), respectively.

**Polysaccharides**

The polysaccharide materials were indicated as magenta colour with PAS stain, the average of carbohydrates in the control lung recorded 1.028 (Fig. 3 D). The treatment with low doses of diazinon induced significant reduction in the polysaccharides content in TII (Fig. 3 E) and TIII (Fig. 3 F) while in TI this reduction was non-significant. The percentages of these reduction recorded 9.34, 24.81 and 28.79% in TI, TII and TIII, respectively (Table 2). The average of polysaccharides in the intestine of the control animals recorded 1.866 (Fig. 4 D). This average recorded significant reduction in all diazinon-treated groups in comparison with the control. The percentage of change reached -46.94 in TII (Fig. 4 E) and -63.29% in TIII (Fig. 4 F).

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**Figure 2**

- **A** - T.S. of control guinea pig small intestine showing normal villi (V). (H&E stain).
- **B** - T.S. of the intestine of TI showing infiltration of lymphocytes within the villi (arrows). (H&E stain).
- **C** - T.S. of the intestine of TII showing haemorrage in the duodenal submucosa and mucosa (H) and erosion in the mucosa (arrow) and disarray in the villi. (H&E stain).
- **D** - T.S. of the intestine of TII showing erosion (tall arrow) and aggregation of lymphocytes (short arrow). (H&E stain).
- **E** - T.S. of the intestine of TIII showing disarray in the villi pattern (arrows). (H&E stain).
- **F** - T.S. of the intestine of TIII showing pynknosis (P), necrosis (N) and degenerated area (D) in the intestinal crypts. (H&E stain).
Table 2 - Mean # optical density (M.O.D.) values relative to histochemical reactions in the lung and intestine of control and treated animals.

<table>
<thead>
<tr>
<th></th>
<th>Total Proteins</th>
<th>Polysaccharides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>TI</td>
</tr>
<tr>
<td><strong>Lung</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>1.576</td>
<td>1.408</td>
</tr>
<tr>
<td>SD</td>
<td>0.408</td>
<td>0.390</td>
</tr>
<tr>
<td>SE</td>
<td>0.166</td>
<td>0.159</td>
</tr>
<tr>
<td>t test</td>
<td>0.2076</td>
<td>0.034</td>
</tr>
<tr>
<td>Percentage of change</td>
<td>-10.66%</td>
<td>-36.55%</td>
</tr>
<tr>
<td><strong>Intestine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>2.066</td>
<td>1.423</td>
</tr>
<tr>
<td>SD</td>
<td>0.393</td>
<td>0.489</td>
</tr>
<tr>
<td>SE</td>
<td>0.160</td>
<td>0.199</td>
</tr>
<tr>
<td>t test</td>
<td>0.0052</td>
<td>0.0055</td>
</tr>
<tr>
<td>Percentage of change</td>
<td>-31.12%</td>
<td>-45.15%</td>
</tr>
</tbody>
</table>

Figure 3 - A - T. S. of control guinea pig lung showing normal content of the total protein (Bromphenol blue stain).
B - T. S. of the lung of TII showing reduction in the total protein content. (Bromphenol blue stain).
C - T. S. of the lung of TIII showing reduction in the total protein content. (Bromphenol blue stain).
D - T. S. of control guinea pig lung showing normal content of the polysaccharides content (PAS stain).
E - T. S. of the lung of TII showing reduction in the polysaccharides content (PAS stain).
F - T. S. of the lung of TIII showing reduction in the carbohydrates content (PAS stain).
DISCUSSION

The exposure to diazinon is a complicated subject. The organophosphates are efficiently absorbed by inhalation, ingestion and skin penetration and this exposure by the multiple routes can lead to serious additive toxicity (Reigart and Roberts, 1999). The LD$_{50}$ value of diazinon orally administration in male guinea pigs (Cavia porcellus) recorded in the present study was 213 mg/kg body weight. Similarly, the LD$_{50}$ calculated was close to that obtained by Worthing and Hance (1991) i.e. 210 mg/kg B W of guinea pig and WHO (1999) i.e. 200 mg/kg B W of rat. Otherwise, the LD$_{50}$ was 300 to 400 mg/kg for technical grade diazinon in rats (Gallo and Lawryk, 1991).

However, considering the classification of the toxicity of the substances (Matsumura, 1975), extremely toxic substance had LD$_{50}$ less than 1mg/kg, highly toxic substance from 1-50 mg/kg, moderately toxic substance between 50-500 mg/kg and relatively harmless 15 g/kg and above. Based on this classification, diazinon appeared slightly toxic in the male guinea pig using oral route of administration. The symptoms that appeared here were indicative of the toxicity of diazinon on the central nervous system in accordance with Dede (1992). Also, the convulsion as a neurological sign recorded in most animals in the current study was
in agreement with Smith (1991) and Dede and Dogara (2004). In this respect, Manna et al. (2004a) attributed the convulsion, produced due to the exposure to insecticides, to the enhancement in lactic acid production. The increased respiratory rate could be a direct result of lung reaction to diazinon (Dorland, 1977) or the effect of the chemical on the respiratory centre in the reticular formation (Dede and Simini 2001). However, Gallo and Lawryk, (1991) reported that in the animals, diazinon converted to diazoxon which was a strong cholinesterase inhibitor.

The xenobiotics can adversely affect the lung, resulting in respiratory insufficiency (Guyton, 1976). The present results showed that the exposure to the sub lethal doses of diazinon insecticide induced dose-dependent histopathological lesions in the lung of guinea pig. These lesions were mostly represented in the infiltration of the macrophages and mononuclear cells, haemorrhage, congested blood vessels, oedema, pyknosis and necrosis. Then, alterations in the lung tissue observed in the present investigation, as a consequence of diazinon insecticide application, showed similarity and conforms those recorded by several authors in various experimental animals exposed to organophosphorus insecticides (Williams and Sandler, 2001; Alavanja et al., 2004 and Hemmati et al., 2005). Giray et al. (2001) discussed that such these histopathological changes could be attributed to the decreases in the antioxidant status of the animal body induced by the chemical.

The congestion of the lung vasculature which was noticed in the present investigation, could be due to the excessive blood in venous system, which increased blood pressure in the veins and capillaries. This might exert undue pressure on the neighboring structures. This is usually accompanied be a diminished blood supply, thus become subjected to malnutrition, deficient oxygenation and the accumulation of the excretory products (Haschek and Rousseaux, 1991).

Latuszynska et al. (1999) showed that the dermal application of the chlorpyrifos, organophosphorus insecticide, induced the foci of various sizes consisting of lymphatic tissue and foam cells in the interalveolar septa in rats. Fryer et al. (2004) demonstrated that the organophosphate insecticides could cause airway hyperactivity in the absence of AChE inhibition by decreasing neuronal receptor function. Alavanja et al. (2004) reported that different pesticides including diazinon have been found to be significantly associated with the lung cancer. Results of the present study revealed that low doses of diazinon insecticide caused different histopathological changes in the small intestine of male guinea pig. These changes were manifested in infiltration and hypertrophy of the lymphocytes, haemorrhage in the submucosa, erosion in lining epithelium, pyknotic nuclei and necrotic cells. These lesions were more evident with the high doses. Similarly, desquamation, haemorrhage and necrosis of the epithelial cells of the stomach and intestine were noticed post α-cypermethrin insecticide oral administration in rats (Manna et al., 2004 a). In vitro study showed that the cultured intestinal and colonic cell proliferation was decreased by diazinon insecticide (Greenman et al., 1997). In contrary of the present results, Zaleska-Freljan et al. (1983) reported that promfenvinphos organophosphate pesticide induced no changes in the stomach, small and large intestine of mice.

It has been suggested that the tissue proteins and polysaccharides were generally inhibited in various animals to which organophosphate was applied. The present results indicated that low doses of diazinon caused significant reduction in both the proteins and carbohydrates in a dose dependent manner in the lung and small intestine of guinea pig. Ritter (1977) reported that the cell necrosis could be either due to progressive degenerative action of the intracellular enzymes of the injured cells or to a metabolic disturbance and inhibition of synthesis needed for the DNA and hence the protein synthesis for the growth and maturation of the cells. Disturbed carbohydrates metabolism following diazinon treatment was also noticed by Lomte and Mule (1992). Sheela and Muniandy (1992) reported significant decreases in the protein, carbohydrate and lipid content of muscle and liver at different sub lethal concentrations of the organophosphate insecticide dimethoate in rats. Anusha-Amali et al. (1996) revealed that the sub lethal effects of organophosphorus pesticide quinalphos induced the depletion in carbohydrate, protein and lipid contents in all the tissues and this depletion was observed to be concentration dependent.

Generally, the reduction in the carbohydrate and protein contents observed in the present study could be due to the release of the hydrolytic enzymes from ruptured lysosomes under the effect...
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