Histological and Biochemical Effects of Diazinon on Liver and Kidney of Rabbits

O.M.M.Sarhan[1,2] and Z.Y. Al-Sahhaf[1]

[1]Department of Biology, Faculty of Applied Sciences, Umm-Al-Qura University, Makkah Al-Mukarammah, Saudi Arabia
[2]Department of Zoology, Faculty of Sciences, Fayoum University, Egypt
Corresponding Author: Osama M. M. Sarhan
omsarhan@hotmail.com

Abstract: The present study was carried out to investigate the effect of diazinon on histological and biochemical aspect of liver and kidney of rabbit. Diazinon induced blood vessel congestion, leucocytic infiltrations in the liver parenchyma in addition to cytoplasmic vacuolation, fatty degeneration and pyknotic nuclei in the hepatocytes. On the other hand, renal damage was observed in the kidneys of treated rabbits. Renal tissues showed hypertrophied glomeruli, destructive of its lining epithelia. Renal blood vessels were congested and the inter-tubular spaces were filled with red blood cells. Biochemical investigation proved that treatment with diazinon for 4 weeks induced a significant increase in ALT, AST, creatinine and blood urea. Finally, the investigators concluded that diazinon toxicity induced hepatocellular and renal damage.

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Key Words: Diazinon, liver, kidney, rabbit, histology and biochemistry.

1. Introduction

Pesticides are synthetic organic compounds that are deliberately introduced into the environment to control selected organisms. Contact with organophosphorus pesticides is an important health problem for agricultural workers (Hurtig *et al*., 2003). Some of these pesticides are highly toxic for mammals (Abdel-Salam and Ford, 1987; El-Shenawy *et al*., 2009). Organophosphorus insecticides are used throughout the world for control of agricultural and domestic insect pests. Diazinon [phosphoric acid, O, O-diethyl O (2-isopropyl-6-methyl-4-pyridinyl)] phosphorothioate is an organophosphorus insecticide widely used in agricultural practice throughout the world to control flies, lice, and other insect pests of ornamental plants and food crops (Johnson and Hanstbarger, 1966). Due to extensive use of diazinon, its residues have been detected in foodstuffs designed for human consumption (Johnson and Manske, 1977). The toxic effects of diazinon on animals were studied by some investigators (Abdou and ElMazoudy, 2010; Shah and Iqbal, 2010). Ceron *et al*. (1996) reported that diazinon inhibits acetylcholinesterase activity and other organic functions. Diazinon was also found to lead to alterations in blood factors, plasma testosterone and glucose levels in male rats (Alahyary *et al*., 2008). Oral administration of diazinon to mice resulted in decrease in splenic T-dependent antibody response to DNP-ficoll and a dramatic thymus atrophy (Kump *et al*., 1996). Other studies have indicated that diazinon has the capacity to disrupt reproductive function in animals (Rodriguez and Bustos-Obregn, 2000 and Yehia et *al*., 2007). Gokcimen *et al*. (2007) reported that diazinon induced histopathological changes in liver and pancreas of rats. Diazinon treatment induced hematological changes (Kalender *et al*., 2006) as well as hepatotoxicity (Kalender *et al*., 2005) in rats. The present work aims to investigate the histological and biochemical effects of diazinon on liver and kidney of rabbits.

Materials and Methods

Animals and treatment

15 Male New Zealand white rabbits weighing 1.8-2 kg were housed in the laboratory at controlled light and temperature. They were provided with rabbit chow and fresh water. Animals were divided equally into 3 groups:

Group 1. Animals of this group were considered as controls.

Group 2. Animals of this group were given diazinon in drinking water at a dose level of 20 mg/kg body weight, every 48 hrs for 2 weeks.

Group 3. Animals of this group were given diazinon in drinking water at a dose level of 20 mg/kg body weight, every 48 hrs for 4 weeks. Diazinon was applied as commercial emulsifiable concentrate formulation containing 60% active ingredient, then, it was further diluted in distilled water to obtain the desired concentration.

Histological examination

The treated animals and their controls were killed, quickly dissected and their liver and kidney were removed, sliced and fixed in Bouin's fluid. After 24 h, tissues were rinsed three times in 70% ethanol, dehydrated using a graded ethanol series and then embedded in paraffin wax. Paraffin sections were cut

into 5 micrometers thick slices and stained with haematoxylin and eosin and examined under light microscope.

Biochemical assays

Blood was collected from controls and treated animals after 2and 4 weeks of treatment. For biochemical study sera were obtained by centrifugation then creatinine and urea were determined using Henry's methods (1974). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically according to Reitman and Frankel (1957).

3. Results

Histological results:

Figure 1 showed histological structure of liver in control rabbit. The liver is divided into hepatic lobules formed of radially arranged strands of hepatocytes that extend from the central vein to periphery of the lobule. The hepatocytes strands are separated from each other by blood sinusoids that are lined with the endothelial cells and Kupffer cells. Treating animals with diazinon caused several histopathological alterations. After 2 weeks of treatment (group 2), the blood vessels were congested and the sinusoidal spaces were filled with blood (Fig. 2). The hepatocytes plates were disrupted and the cells were swollen and vacuolated (Fig. 3). Focal inflammatory cells infiltration was abundant (Fig.4). These histopathological alterations were severed in animals examined after 4 weeks (group 3). The blood vessels, central and portal veins were severely congested and bile ducts were degenerated (Fig. 5). The sinusoidal spaces were dilated and filled with red blood cells. The hepatocytes appeared with cytoplasmic vacuolation and pyknotic nuclei (Fig. 6). Some signs of fatty degeneration were observed (Fig. 7).

Histological examination of the kidney of control rabbit revealed normal histological features, illustrated in figure (8). The administration of diazinon caused many histological damage to the renal cortex. Examination of the kidney sections of animals after treatment with diazinon for 2 weeks (group 2), showed that renal blood vessels were congested (Fig. 9). Most of renal tubules were damaged and lost their characteristic appearance and their lining epithelial cells were destructed. The glomeruli were hypertrophied (Fig. 10). After 4 weeks (group 3), the renal tubules were severely damaged and their cells showed cytoplasmic vacuolation and some glomeruli were atrophied (Fig. 11). Blood capillaries in between the degenerated tubules were congested (Fig. 12).

Biochemical results

Treating animals with diazinon induced an increase in ALT and AST. This increase became significant ($P<0.05$) after 4 weeks of treatment (Figs.13&14). Similarly, creatinine and blood urea increased significantly ($P<0.05$) in the rabbits determined after 4 weeks of treatment with diazinon (Figs.15&16).

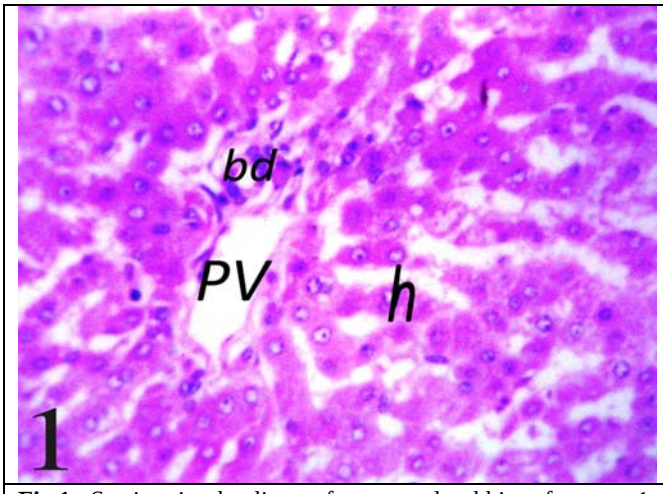

Fig.1. Section in the liver of a control rabbit, of group 1, showing portal vein (PV) and bile duct (bd) in the portal area and surrounding hepatocytes (h), (X 200).

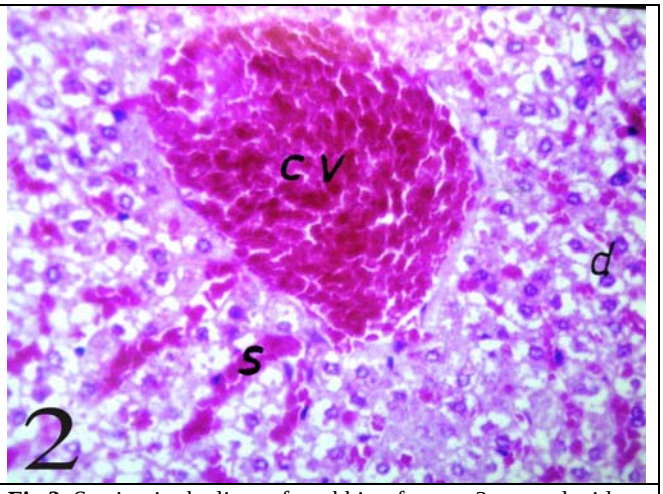

Fig.2. Section in the liver of a rabbit, of group 2, treated with diazinon for 2 weeks showing severe congestion in central vein (CV) and sinusoids (S) with degeneration in the hepatocytes (d), (X 200).

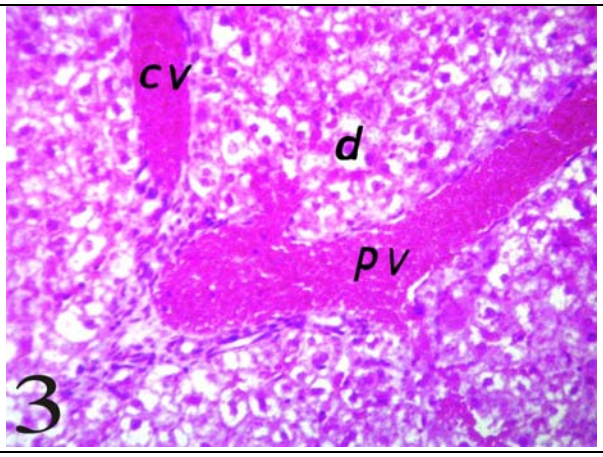

Fig.3. Section in the liver of a rabbit, of group 2, treated with diazinon for 2 weeks showing congestion of central (CV) and portal (PV) veins. The degenerated hepatocytes (d) showed cytoplasmic vacuolation, (X 200).

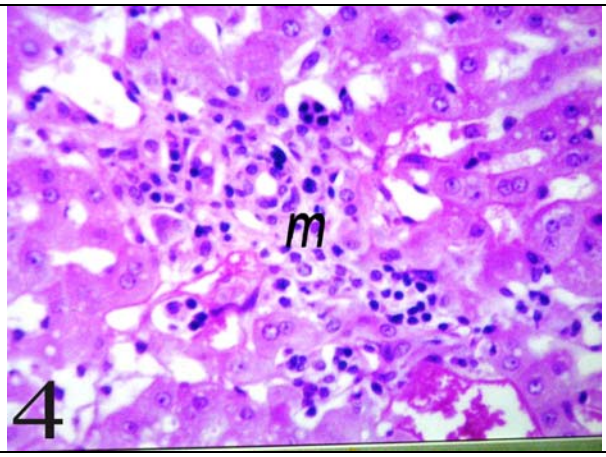

Fig.4. Section in the liver of a rabbit, of group 2, treated with diazinon for 2 weeks showing focal inflammatory cells infiltration (m) in hepatic parenchyma, (X 200).

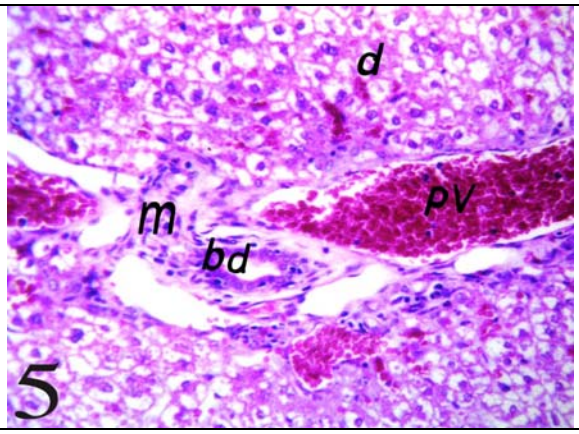

Fig.5. Section in the liver of a rabbit, of group 3, treated with diazinon for 4 weeks showing degenerated hepatocytes (d), congestion in portal vein (PV), inflammatory cells infiltration (m) and degenerated bile duct (bd), (X 120).

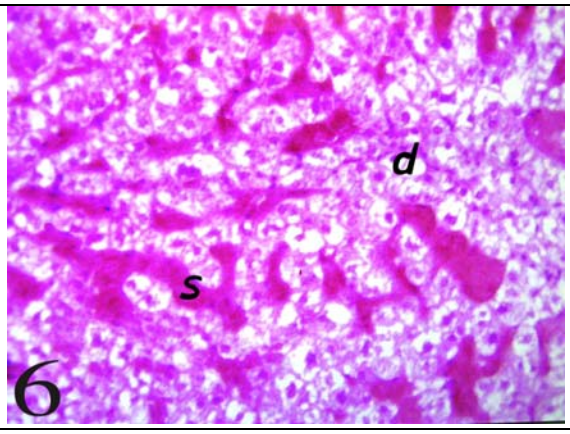

Fig.6. Section in the liver of a rabbit, of group 3, treated with diazinon for 4 weeks showing degenerated hepatocytes (d) and severe congestion in the sinusoids (S) between them, (X 120).

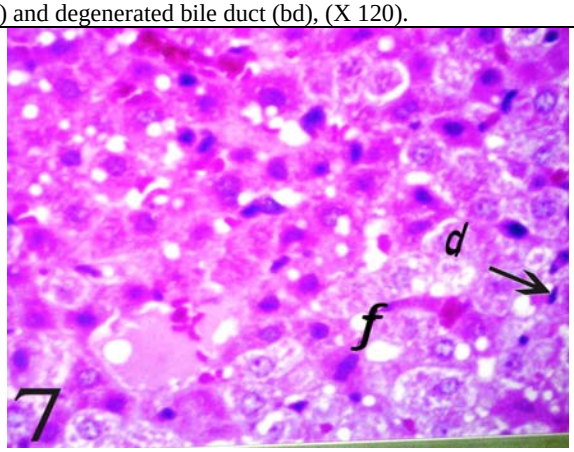

Fig.7. Section in the liver of a rabbit, of group 3, treated with diazinon for 4 weeks showing degenerated hepatocytes (d), fatty degeneration (f) and activated Kupffer cells (arrow), (X 200).

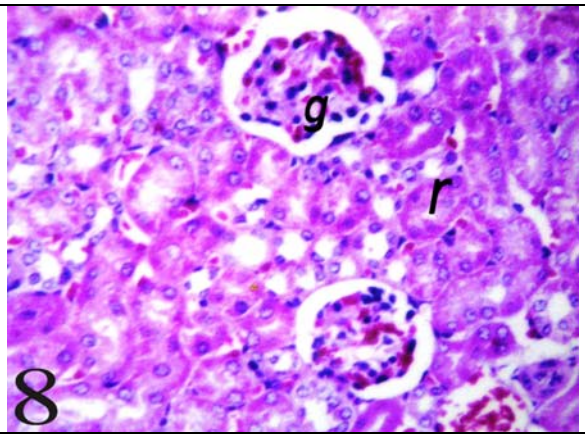

Fig.8. Section in the kidney of a control rabbit showing normal renal tubules (r) and glomerulus (g), (X 200).

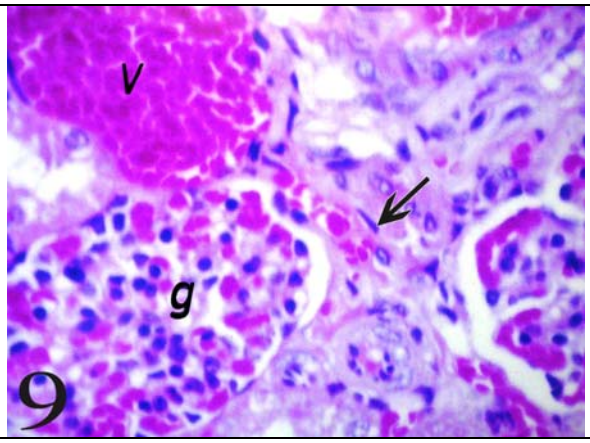

Fig.9. Section in the kidney of a rabbit, of group 2, treated with diazinon for 2 weeks showing enlarged and congested renal vein (V), congested glomerulus (g), and intertubular fibrosis (arrow), (X 200).

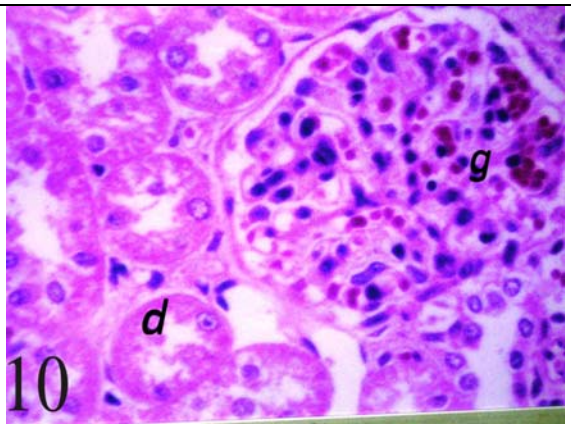

Fig.10. Section in the kidney of a rabbit, of group 2, treated with diazinon for 2 weeks showing hypertrophy, proliferation and swelling in the lining endothelium of the glomerulus tuft (g) with degeneration in the lining epithelial cells of renal tubules (d), (X 200).

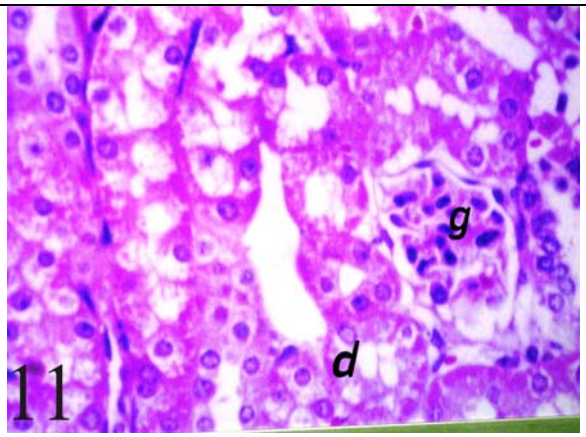

Fig.11. Section in the kidney of a rabbit, of group 3, treated with diazinon for 4 weeks showing atrophy of a glomerulus (g) with degeneration in the lining epithelial cells of renal tubules (d), (X 200).

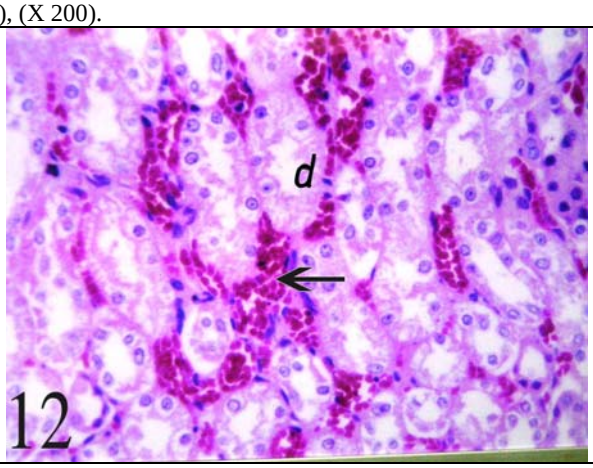

Fig.12. Section in the kidney of a rabbit, of group 3, treated with diazinon for 4 weeks showing congestion in blood capillaries (arrow) in between the degenerated renal tubules (d), (X200).

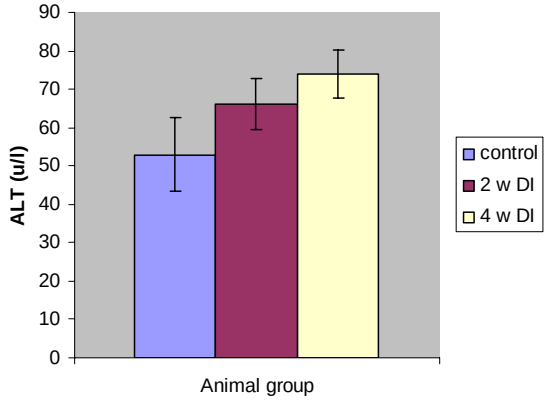

Fig.13. Effect of treatment with diazinon (DI) on ALT.

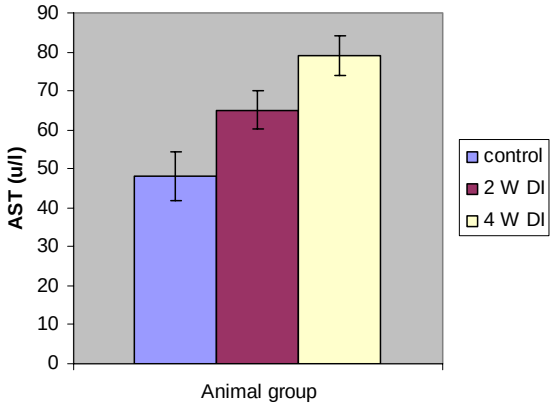

Fig.14. Effect of treatment with diazinon (DI) on AST.

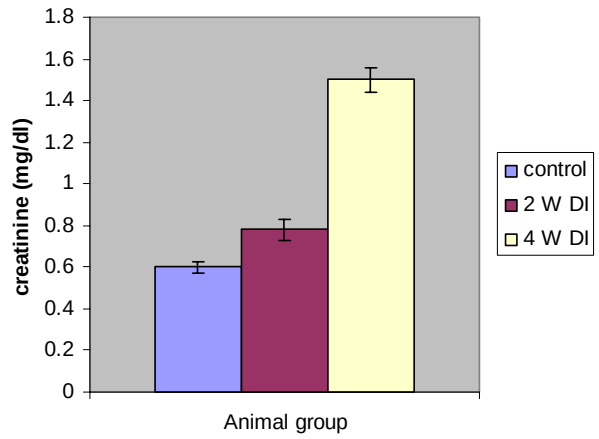

Fig.15. Effect of treatment with diazinon (DI) on creatinine.

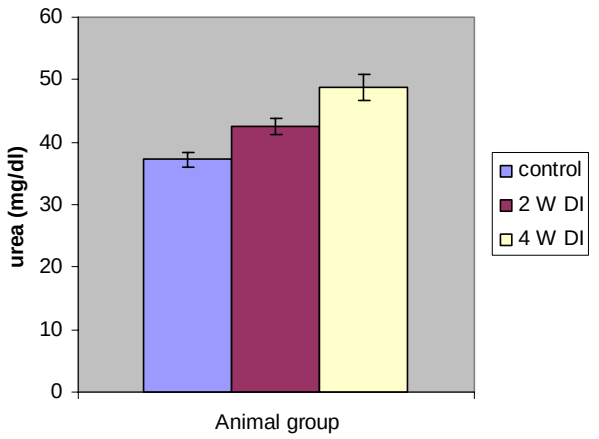

Fig.16. Effect of treatment with diazinon (DI) on serum urea.

4. Discussion

Results of the present work indicated that diazinon induced histopathological alterations in the liver of rabbit. The liver showed congestion of veins, leucocytic infiltrations, cytoplasmic vacuolation of the hepatocytes and fatty degeneration. Similarly, El-Shenawy *et al*. (2009) reported that intoxicated mice with diazinon resulted in hydropic degeneration, necrosis and focal microvesicular steatosis in liver. Jacqueson *et al.* (1977) and Anthony *et al*. (1986) observed that the liver of male Wistar rats chronically treated with sublethal doses of diazinon sustain a form of hepatic injury characterized by cellular lipid accumulation. Abdel-Salam and Ford (1987) showed that diazinon induced liver and kidney damage in ruminant live stock. They added that this hepatotoxic effect was enhanced by carbon tetrachloride, while the renal toxic effect was enhanced by mercuric chloride. Hyperplasia of hepatocytes, necrosis, lymphocytic infiltrations and steatosis were observed in rats treated with 1/20 LC_{50} of diazinon (Hassan *et al*., 2007). Cytoplasmic vacuolations were observed in hepatocytes of diazinon-treated animals. It is considered as a form of cell injury which is most frequent in parenchymal cells of liver with wide network of internal membranes that are concerned with ions pumping (Mori, 1987). Cytoplasmic vacuoles develop due to accumulation of ions and water in cytosol and rapidly pass through leaky membranes of cell organelles. Massive accumulation of fluids in the vacuoles may finally lead to cell lysis (Gores *et al*., 1990).

AST and ALT showed a significant increase in rabbits given diazinon. In agreement with these results, Ahmed (2006) who found elevation of transaminases (AST, ALT) in rats treated with 1/30 LD_{50} diazinon for 3 weeks. Kalender *et al.* (2005) recorded an elevation in ALT, AST, ALP, total cholesterol, and triglyceride levels in rats treated with diazinon. Transaminases were considered to be a more sensitive measure in evaluating liver function and damage (Sherlock, 1981). Hatoff and Hardison (1980) reported that elevations in serum levels of these enzymes were mostly attributed to acute hepatocellular damage or extrahepatic obstruction, or both.

The present results showed that diazinon treatment led to degeneration of renal tubules, hypertrophy of glomeruli and leucocytic infiltrations. These results indicated that diazinon metabolites caused toxicity in renal system; and the immune system makes a good role for defending against foreign particles. The effect of diazinon on kidney was studied in different animals. Oral administration of diazinon for 2 months to male albino rats showed degeneration of the renal tubules (Hassan *et al*., 2007). El-Shenawy *et al*. (2009) reported that exposing mice to diazinon caused degeneration of renal tubules, atrophy of glomeruli and interstitial inflammatory cells infiltrations.

The present results revealed also a significant increase in serum creatinine and urea in response to diazinon toxicity. Similar results were obtained by El-Shenawy *et al*. (2009) in mice. Diazinon (1/30 LD_{50}) markedly decreased serum urea but did not affect creatinine level (Ahmed, 2006). The increase in creatinine recorded in this work might be due to impaired kidney function by the used fungicide. This view was supported by Kluwe (1981) who indicated that an elevation of creatinine level in the blood is an indicative of impaired kidney function.

Toxicity of diazinon is realized through the inhibition of enzyme acetylcholinesterase whose biological role is the termination of impulse transmissions at cholinergic synapses within the nervous system by rapid hydrolysis of the neurotransmitter -acetylcholine (Schumacher *et al*., 1986). Other studies reported that oxidative stress plays

an important role in the toxicity of diazinon. Malondialdehyde is an indicator of lipid peroxidation, free radical generation and oxidative stress. Catalase and superoxide dismutase are antioxidant enzymes. Diazinon was found to increase malondialdehyde level and decrease antioxidant enzymes in rat erythrocytes (Sutcu *et al*., 2007). Treatment of rats with diazinon significantly enhances renal lipid peroxidation which is accompanied by a decrease in the activities of renal antioxidant enzymes (e.g. catalase, glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase, glutathione S-transferase) and a depletion in the level of glutathione reduced (Shah and Iqbal, 2010). Abdou and ElMazoudy (2010) reported that diazionninduced significant increases in the level of serum malondialdehyde and the activity of lactate dehydrogenase in female rats. Treatment with diazinon induced significant increase in lipid peroxidation and decreased total antioxidant capacity in rat liver and muscle (Amirkabirian *et al*., 2007). The observed hepatotoxicity and nephrotoxicity in the present work may be attributed to oxidative stress generated by diazinon.

Correspondence author name: Osama M.M. Sarhan
omsarhan@hotmail.com

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