



HISTOLOGICAL EFFECTS OF INSECTICIDE PROTHOR (IMIDACLOPRID) IN THE WHITE MALE MICE

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ABSTRACT

A histological study was conducted to investigate the effects of insecticide Imidacloprid (IMD) on the three organs (liver, kidney and spleen) of white adult male mice under two experiments: 1st experiment (sub chronic effects), carried out in order to determine the acceptable daily intake (ADI), 0.06 mg/kg for body weight, with different concentrations 0.11 and 0.17 mg/kg.bw. The effects have been examined after 14 and 28 days of daily oral administration. 2nd experiment (chronic effects), histological study of the present aimed to determine the effects of concentrations less than (ADI) (0.03 mg/kg.bw of daily oral administration). The effects have been examined after 60 and 90 days on the same organs. Results revealed that the effects of insecticide Imidacloprid on mice liver are infiltration of inflammatory cells, depletion of glycoprotein, hydropic degeneration and hemorrhage after 14, 28, 60 and 90 days at different concentrations 0.06, 0.11, 0.17 and 0.03 mg/kg.bw, while kidney and spleen did not show changes after 14 and 60 days at all doses however changes were detected after 28 and 90 days.

KEYWORDS: Imidacloprid, white mice, nicotine.

INTRODUCTION

Pesticides are chemical substances designed to kill or inhibit the growth of undesirable organisms, usually in a selective manner. Pesticides are considered as any substances preparation or microorganism prepared to use for protecting and regulating the growth of plants, protecting animals against ectoparasites (Anon, 2000). Pesticides are extensively used all over the world since the human realization to control the harmful effects of pests on different crops and food stuffs to cater maximum yield of growing human population (Ajay *et al.*, 2014). Imidacloprid (IMD) was launched in 1991 for the first time by Bayer Crop-Science (Elbert, 1990). Imidacloprid is an extensively used for crop protection worldwide from the last decade due to its low soil persistence and high insecticidal activity at very low application rate (Elbert, 2007). The selective toxicity of Imidacloprid to insects and not to mammals reported to be due to differences in the structure and binding affinity at the nicotinic acetylcholine receptor (Tomizawa *et al.*, 2003). Imidacloprid exposure leads to block a type of neuronal pathway which causes the accumulation of acetylcholine which leads to paralysis and eventually death of the insect pests (Wang *et al.*, 2012).

MATERIALS & METHODS

Insecticide

Present study used insecticide (Prothor) which obtained from Baghdad markets as commercial formulation form manufactured by EnsystexII, Inc, Fayetteville, NC, with infective material Imidacloprid 20%.

Animals

Eighty four Swiss male mice, 12-14 weeks ages and 25-30g body weight. Mice were obtained from the National Center of Drug Control and Researches. They were kept in

a room, supplied with normal conditions to keep the temperature at 24°C.

Experimental design:

1st experiment (sub chronic effects)

Eighty four mice were divided into four groups each group contain 12 mice.

G 1: Inoculated orally with normal saline (0.1 ml), considered as control.

G 2: Inoculated pesticide (0.1 ml) with dose of (0.06 mg/kg.bw) scarified after 14 days and then after 28 days.

G 3: Inoculated pesticide (0.1 ml) with dose of (0.11 mg/kg.bw) scarified after 14 days and then after 28 days.

G4: Inoculated pesticide (0.1 ml) with dose of (0.17 mg/kg.bw) scarified after 14 days and then after 28 days.

2nd experiment (chronic effects):

Thirty six mice were divided into two groups:

G5: Twenty four Inoculated pesticide (0.1 ml) with dose of (0.03 mg/kg.bw) scarified after 60 days and then after 90 days (12 mice Inoculated orally with normal saline considered as control). From all mice groups, the organs liver, kidney and spleen that saved in formalin 10% then stain with Eosin and haematoxylin stains taken to study histological changes.

RESULTS & DISCUSSION

The results of the current study showed that many changes ranged from mild to severe effects were shown that depending on the concentration dose and time. The control group shows the normal shape of mice organs (liver, kidney and spleen) as shown in figure (1-A, B, C), whereas, in group (2) the liver shows infiltration of inflammatory cells and depletion of glycoprotein after 14 days (Figure 2-A), while kidney and spleen did not reveal changes, however after 28 days the liver show hydropic degeneration (Figure 2-B). The kidney shows hydropic

degeneration of urinary epithelial cell (Figure 2-C) and spleen show increasing in the number of megakaryocytes (Figure 2-D). In group (3) the liver show changes as depletion of glycoprotein and hemorrhage after 14 days (Figure 3-A). The kidney and spleen did not show any changes, while after 28 days the liver show sever hemorrhage, infiltration of lymphocytes and hydropic degeneration (Figure 3-B). The kidney shows congestion (Figure 3-C) and spleen shows an increasing in the number of megakaryocytes (Figure 2-D). Concerning the group (4), the liver shows a hemorrhage after 14 days (Figure 4-A), while no changes were detected in the kidney and spleen, however, after 28 days the liver show necrosis and infiltration of lymphocytes (Figure 4-B). The kidney shows congestion blood vessels with necrosis of epithelial cell lining cells of renal tubules (Figure 4-C) and spleen shows an increasing in the number of megakaryocytes, necrosis and fibroses (Figure 4-D). In regards with the group(5), the liver shows a congestion and sever infiltration of lymphocytes after 60 days (Figure 5-A), while the kidney and spleen didn't show any changes, however after 90 days the liver show necrosis, infiltration of lymphocytes and increasing of number of kuppfer cells (Figure 5-B). The kidney shows a depletion of renal tubules with filtration their lining epithelial cells (Figure 5-C) and the spleen shows an increasing in the number of

megakaryocytes with widening of white pulp and fibroses (Figure 5-D). The toxic effects of IMD indicated that some toxic metabolites may be transported from intestine to liver that caused these changes. Nidhi *et al.* (2006) reported the presence of definite necrosis indicated capability of the toxic metabolites causing cell death. Hassan *et al.* (2007) reported that the necrosis and infiltration of lymphocytes were observed in rats treated with diazinon pesticide. These results agree with our results. The increasing in the extent of the damage with increasing doses of IMD and duration of administrated was noticed in liver structural anatomy. Similar results obtained by Tos-Luty *et al.* (2003) who found histopathological changes in the Wistar rats treated with malathion pesticide and Gokcimen *et al.* (2007) who found histopathological changes in the rats liver exposed to dose of diazinon pesticide. In the kidney, these changes reflected action of toxic metabolites of pesticide (Nidhi *et al.*, 2006). Tos-Luty *et al.* (2003) reported that the malathion pesticide led to an increasing in the extent of the damage associated with increasing in the doses of IMD and duration of administrated and caused necrosis of epithelia cell lining cells of renal tubules the Wistar rats. In spleen these changes may be attributed to loss of infiltration efficiency because of the toxicity of IMD (Balani *et al.*, 2008).

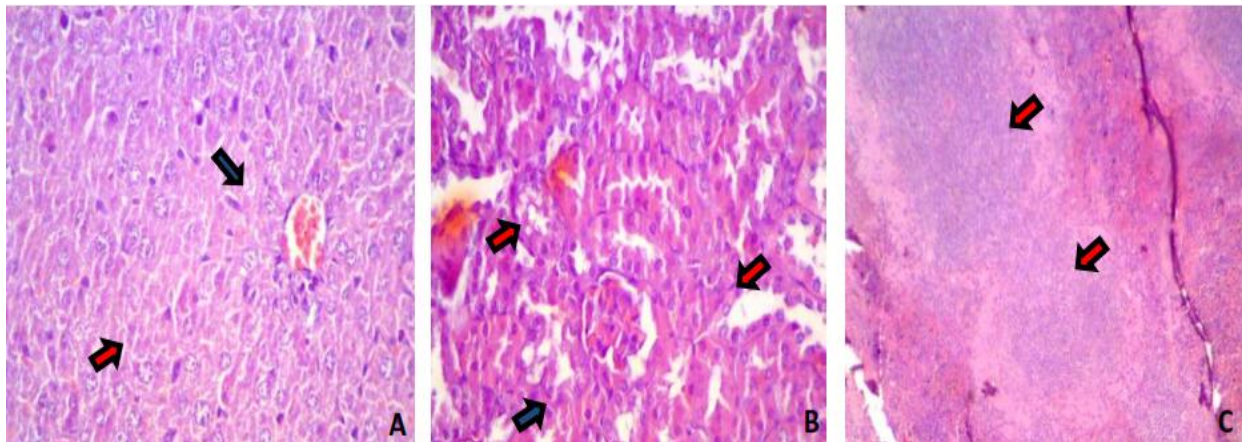
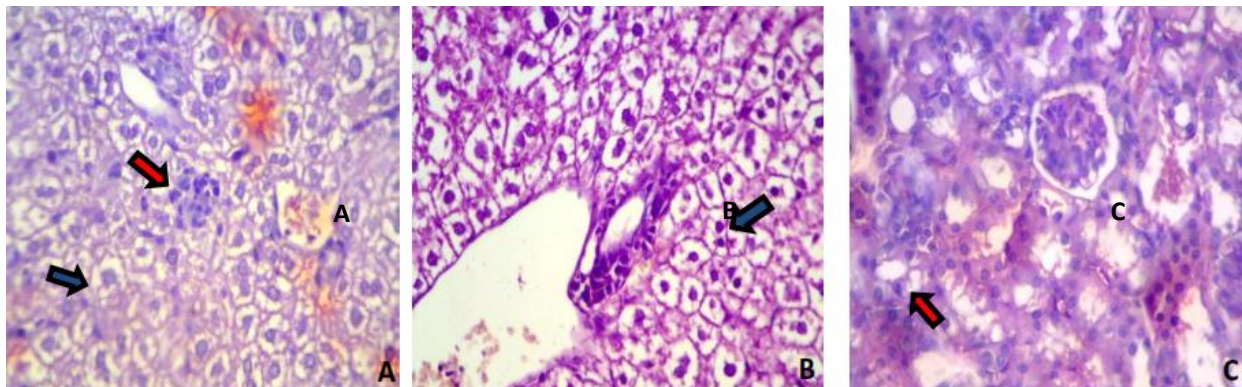


FIGURE 1: A. Cross section in control mice liver showing normal shape compose of central veins (blue arrow) and hepatocytes (red arrow) B. Cross section in control mice kidney showing normal shape composes of glomerulus (blue arrow) and proximal and distal convoluted tubules (red arrow) C. Cross section in control mice spleen showing normal shape composes of white and red pulp (red arrow) 200x stained by H&E stain.



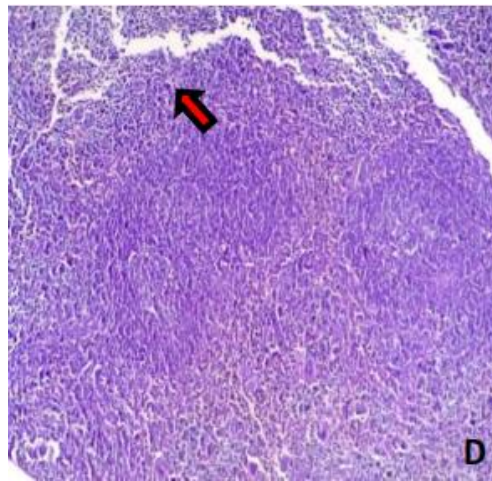


FIGURE 2: **A.** cross section at dose 0.06mg/kg.bw in mice liver at 14 days showing an infiltration of inflammatory cells (red arrow) and depletion of glycoprotein (blue arrow) 200x. **B.** cross section at dose 0.06mg/kg.bw in mice liver at 28 days showing hydropic degeneration (blue arrow) 400x. **C.** cross section at dose 0.06mg/kg.bw in mice kidney at 28 days showing hydropic degeneration of urinary epithelial cell (red arrow) 100x. **D.** cross section at dose (0.06 and 0.11mg/kg.bw) in mice spleen at 28 days showing increasing in number of megakaryocytes (red arrow) 100x stained with H&E stain.

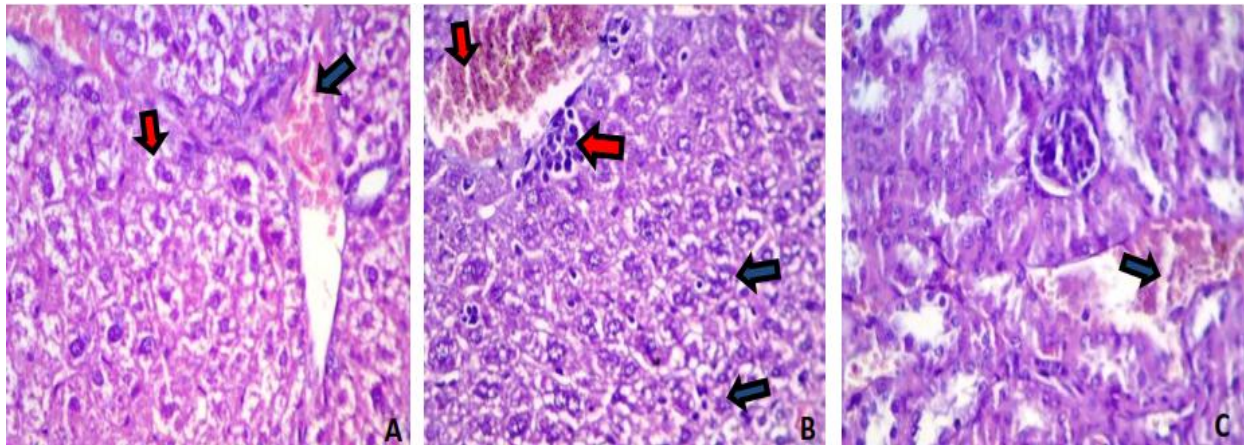
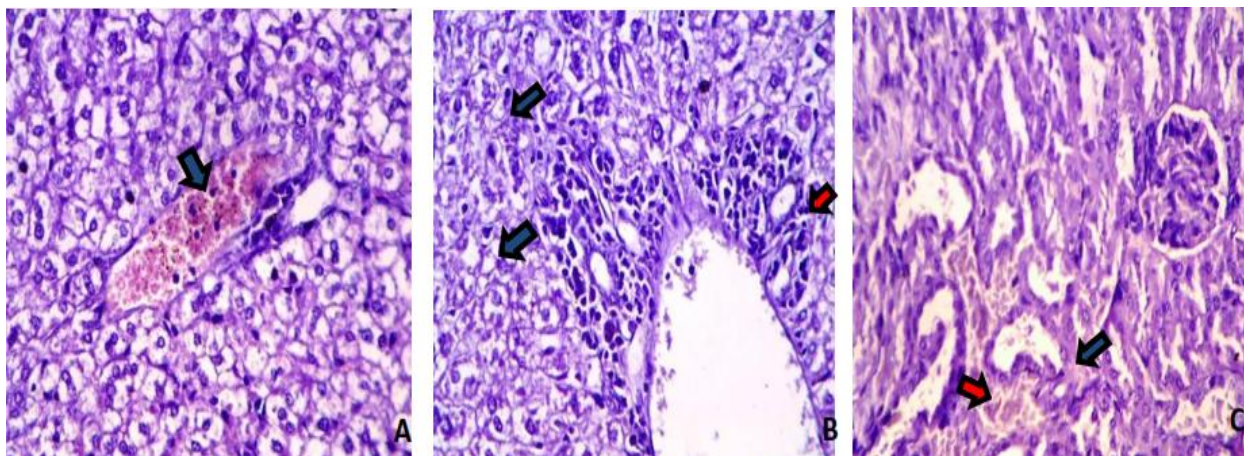


FIGURE 3: **A.** cross section at dose 0.11mg/kg.bw in mice liver at 14 days showing depletion of glycogen (red arrow) and hemorrhage (blue arrow) **B.** cross section at dose 0.11mg/kg.bw in mice liver at 28 days showing severe hemorrhage and infiltration of lymphocytes (red arrow), hydropic degeneration (blue arrow) **C.** cross section at dose 0.11mg/kg.bw in mice kidney at 28 days showing congestion (blue arrow) 200x stained with H&E stain.



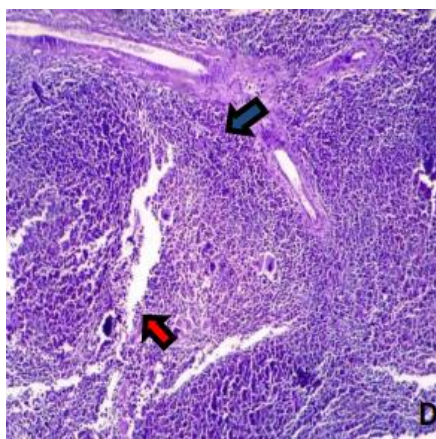


FIGURE 4: **A.** cross section at dose 0.17mg/kg.bw in mice liver at 14 days showing hemorrhage (blue arrow) **B.** cross section at dose 0.17mg/kg.bw in mice liver at 28 days showing necrosis (blue arrow) and infiltration of lymphocytes (red arrow) 200x. **C.** cross section at dose 0.17mg/kg.bw in mice kidney at 28 days showing congestion blood vessels (red arrow) with necrosis of epithelial cell lining cells of renal tubules (blue arrow) 200x **D.** cross section at dose 0.17mg/kg.bw in mice spleen at 28 days showing increasing in number of megakaryocytes (blue arrow), necrosis and fibroses (red arrow).

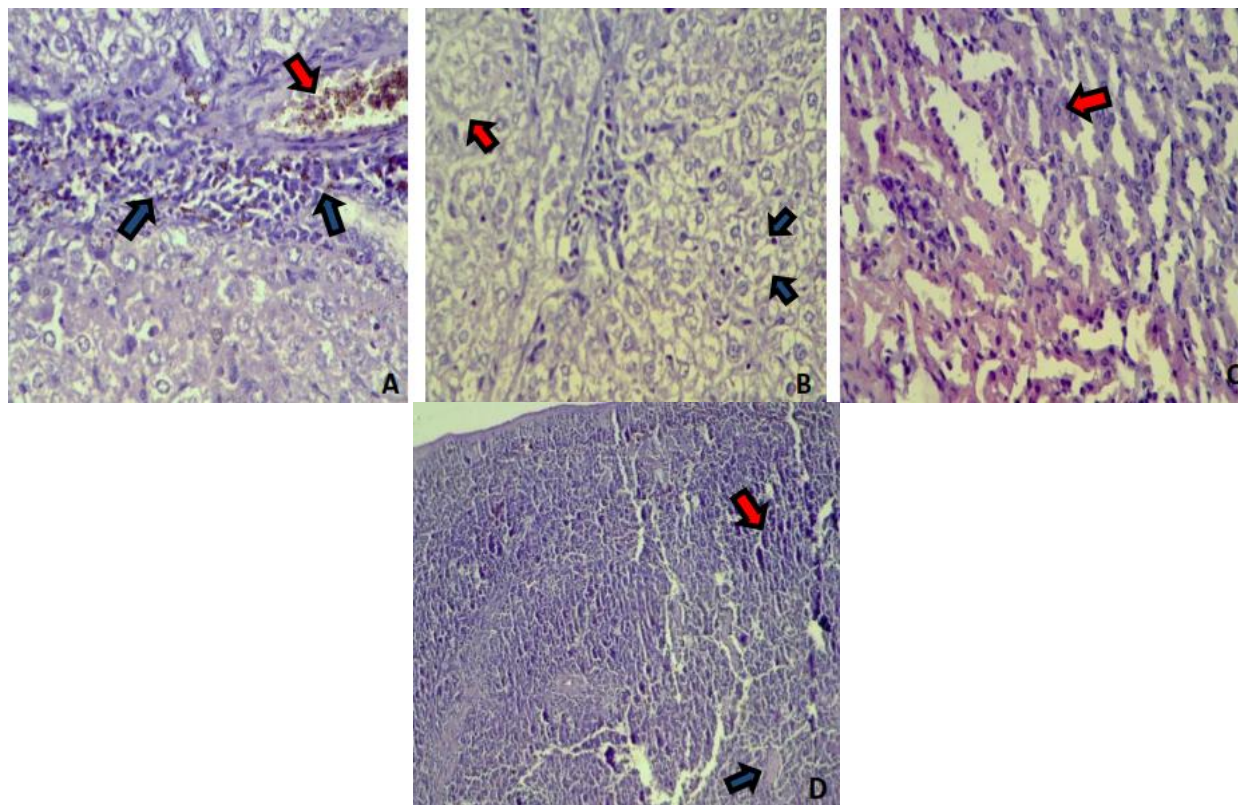


FIGURE 5: **A.** cross section at dose 0.03mg/kg.bw in mice liver at 60 days showing congestion (red arrow) severe infiltration of lymphocytes (blue arrow) 200x **B.** cross section at dose 0.03mg/kg.bw in mice liver at 90 days showing necrosis (red arrow) , infiltration of lymphocytes and increasing of number of kuppfer cells (blue arrow) 200x. **C.** cross section at dose 0.03mg/kg.bw in mice kidney at 90 days showing depletion of renal tubules with filtration their lining epithelial cells (red arrow) 200x. **D.** cross section at dose 0.03mg/kg.bw in mice spleen at 90 days showing increasing of number of megakaryocytes (red arrow) with widening of white pulp and fibroses (blue arrow) 100x stained with H&E stain.

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