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HISTOLOGICAL CHANGES INDUCED BY THE ACTION OF ACTARA 25 WG INSECTICIDES IN MICE

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ABSTRACT
The present study aimed to investigate the histopathological effects of Actara insecticide on albino mice and the traces of this insecticide in liver and kidney. The experiment included 90 mice which were divided into five groups, the first group 10 mice were considered the control animals and the others were divided equally into four groups with a dose of 0.2, 0.4, 0.8, and 1.6 mg / kg of body weight, respectively for a period of 15 and 30 days. The animals given daily via oral route Actara by tube dosage after dissolved with distilled water which was attended to the doses used in the study depending on the value of the Acceptable Daily Intake (ADI) 0.02 mg / kg of body weight in addition to the use of dose comparison. Microscopic examination of liver showed disturbed of the hepatic lobule structure, hepatocytes hypertrophy with severs inflammatory cells infiltration, kupffer cells proliferation, coagulates necrosis and hydropic degeneration. In addition, section of kidney showed lobulated glomeruli, a large area hemorrhage, congested blood vessels that showed thickening in their walls, degeneration changes and infiltration of inflammatory cells. These histological changes were leveled sever according to the dosage and the duration.

KEY WORDS: Actara, histopathology, albino mice, neonicotinoid.

INTRODUCTION

Pesticides are poisonous chemicals that can injure or kill non-target plants and animals or humans and are widely used in agricultural ecosystems (Ecobichon, 2001). Insecticides have a pivotal role in our lives, not only for crop protection in agriculture, but also to avoid the spreading of harmful pests causing human diseases such as malaria (Lopez *et al.,* 2005). Neonicotinoids, the most important new class of synthetic insecticides of the past three decades, are used to control sucking insects both on plants and on companion animals (Tomizawa and Casida, 2005). Neonicotinoids act selectively on insect nicotinic acetylcholine receptors (nAchR), accounting at least in part for the selective toxicity to insects over vertebrates (Tomizawa *et al.,* 2003; Shimomura *et al.,* 2006). Actara is a novel neonicotinoid insecticides belonging to sub class of this nicotinyl compounds and it is systemic insecticide for soil and foliar applications (Tomizawa and Casida, 2005). The product investigated in our experiments - Actara 25WG (thiamethoxam) has been used for the protection of crops and vegetables against bed bugs, the Colorado potato beetle, trips, aphides, flea beetles, whiteflies etc. (Kulkarni and Patil, 2012; Alina *et al*., 2010). Actara a chemical name is: 3-[(2-chloro-5-thiazolyl) methyl] tetrahydro-5-methyl-N-nitro-4H-1, 3, 5-oxadiazin-4 imine. It has as its component the major active ingredient, thiamethoxam 25% (Păunescu *et al.,* 2009). Its mode of action was with contact, stomach and systemic activity (Anikwe *et al.,* 2009). The mode of action is essentially one of cytotoxicity, cell death, both as single cell necrosis and apoptosis, and increased cell replication rates. On the other hand, there was no any available literature concerning histopathological studies of Actara insecticide on mice. Our goal was to describe the histological changes induced by the action of Actara in liver and kidney tissues of albino mice.

MATERIALS AND METHODS

Animals

The animals examined in this study were adult of Balb/c mice of 24±5g were obtained from the Animal Breeding House of Medicine College, Baghdad University. The animals were housed in rooms with 16–20 air-changes per hour, a temperature of 22 ± 2C°, relative humidity of 50 ± 20%, and a 12 h light/ dark cycle. The animals were kept in laboratory condition with tap water for six days to test their health and accommodate them for the experiment. The water was changed daily to avoid the accumulation of toxic substances.

Experimental design

The animals used in the experiment were grouped into five experimental lots as it follows:

- Group of control (1) consisted of 10 of untreated mice, maintained in laboratory conditions in containers with tap water which was changed daily.
- Group (2) consisted of 20 mice, maintained under the same conditions as group 1, 10 of them given daily via oral route Actara in concentration of Acceptable Daily Intake (ADI) (The ADI is the amount of chemical that can be consumed daily for a lifetime without ill effects) 0.2 mg/kg of body weight

(APVMA, 2007) for 15 days and the other half for 30 days with same dosage.

- Group (3) consisted of 20 mice, maintained under the same conditions as group 1, 10 of them given daily via oral route Actara in concentration of 0.4 mg/kg of body weight for 15 days and the other half for 30 days with same dosage.
- Group (4) consisted of 20 mice, maintained under the same conditions as group 1, 10 of them given daily via oral route Actara in concentration of 0.8 mg/kg of body weight for 15 days and the other half for 30 days with same dosage.
- Group (5) consisted of 20 mice, maintained under the same conditions as group 1, 10 of them given daily via oral route Actara in concentration of 1.6 mg/kg of body weight for 15 days and the other half for 30 days with same dosage.

Histopathological Study

We began sacrificing the animals at the end of the experiment; Liver and kidney were quickly removed. The organs were dissected and fixed in 10 % neutral formalin, dehydrated in ascending grades of alcohol and imbedded in paraffin wax. Paraffin sections (5µm thick) were stained for routine histological study using Hematoxylin and Eosin stain (H&E) (Bancroft and Stevens, 1982).

RESULTS

Histopathological effects

Liver: Microscopic examination of liver of control mice showed normal structure of the central vein, hepatocytes and blood sinusoids (Figure 1).

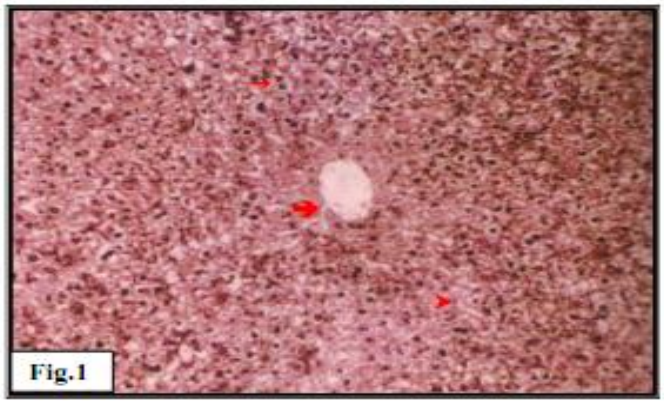

FIGURE 1: A section of liver of control rat show normal structure. Notice the central vein (➔), hepatocytes (→) and blood sinusoids (arrowhead). (H&E X 100).

Histopathological investigation of sections of liver of the treated mice showed in dosage 0. 2 mg /kg body weight for 15 and 30 day after the dosage, aggregation of inflammation cells (Figure 2a) and degeneration changes (Figure 2b). On the other hand, a large area coagulates necrosis (Figure 3a), macrophages infiltration and hepatocytes proliferation (Figure 3b) was observed in dosage 0. 4 mg /kg body weight for 15 and 30 day after the dosage, The histological changes were characterized in dosage 0.8 mg /kg body weight for 15 and 30 day after the dosage, edema with infiltration of inflammatory cells (Figure 4a) and congestion, degeneration of hepatocytes with proliferation of kupffer cells (Figure 4b). The changes increased in high dosage 1.6 mg /kg body weight for 15 and 30 day after dosages which is characterized by sever lymphocytes infiltration (Figure 5a) and disturbed hepatic lobule in the structure of the hydropic degeneration in the hepatocytes and dilation of the hepatic sinusoids was found (Figure 5b).

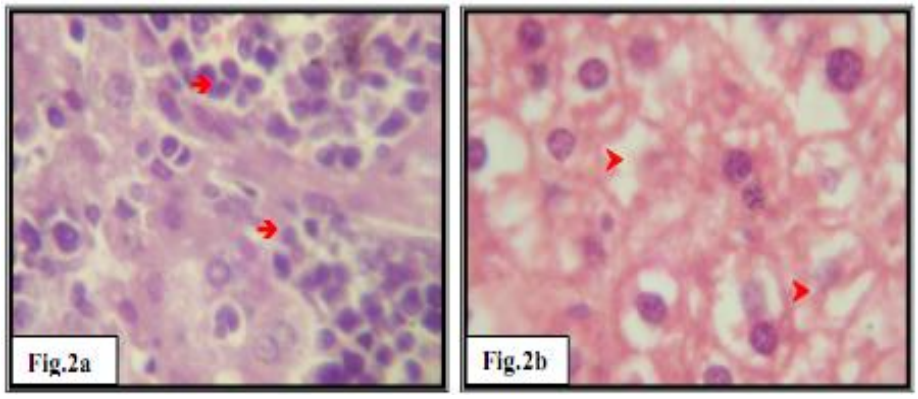

FIGURE 2: A) A section of liver of the treated mice in dosage o.2 mg/kg body weight for 15 day after dosage showing aggregation of inflammation cells (➔). b) A section of liver of the treated mice in dosage o.2 mg/kg body weight for 30 day after dosage showing congestion with degeneration changes (arrowheads).(H&E X400).

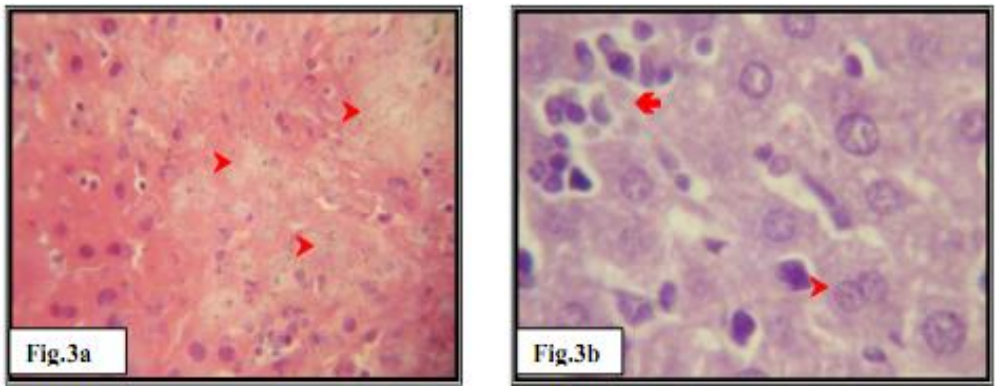

FIGURE 3: A) A section of liver of the treated mice in dosage o.4 mg/kg body weight for 15 day after dosage showing coagulates necrosis with congestion (arrowheads). b) A section of liver of the treated mice in dosage o.4 mg/kg body weight for 30 day after dosage showing infiltration of inflammatory cells (←),hepatocytes proliferation (arrowhead) with degeneration changes.(H&E X400).

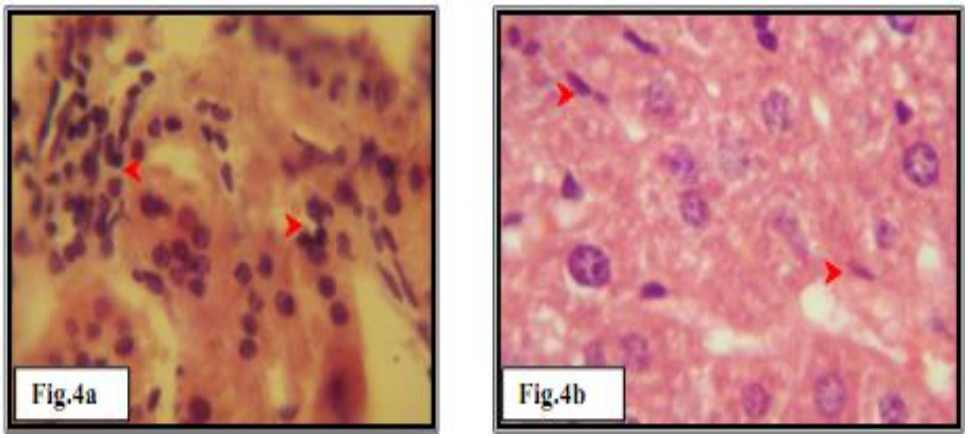

FIGURE 4: a) A section of liver of the treated mice in dosage o.8 mg/kg body weight for 15 day after dosage showing infiltration of inflammatory cells (arrowheads). b) A section of liver of the treated mice in dosage o.8 mg/kg body weight for 30 day after dosage showing congestion, degeneration of hepatocytes with proliferation of kupffer cells (arrowheads), (H&E X400).

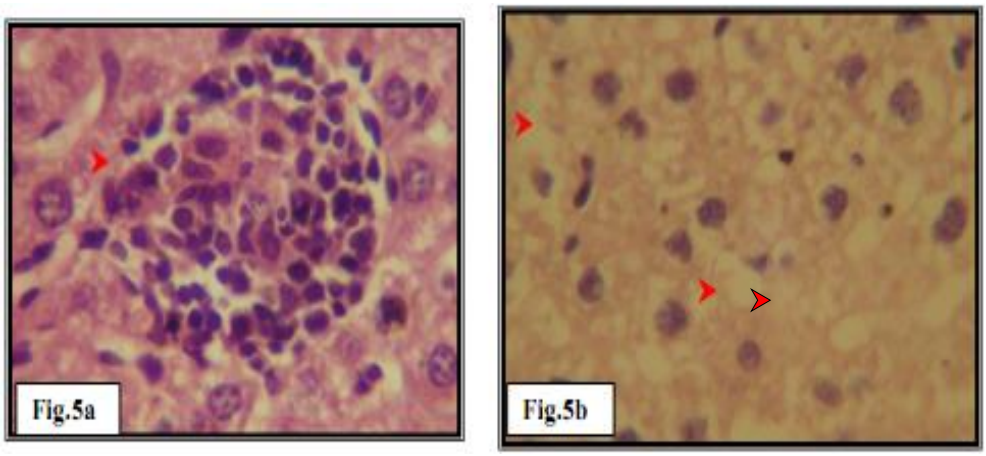

FIGURE 5: a) A section of liver of the treated mice in dosage 1.6 mg/kg body weight for 15 day after dosage showing granulomatous reaction (arrowhead). b) A section of liver of the treated mice in dosage 1.6 mg/kg body weight for 30 day after dosage showing hydropic degeneration in the hepatocytes (arrowheads), (H&E X400).

Kidney: Sections of the kidney of control mice showed normal renal corpuscles, proximal convoluted tubules, and distal convoluted tubules (Figure 6).

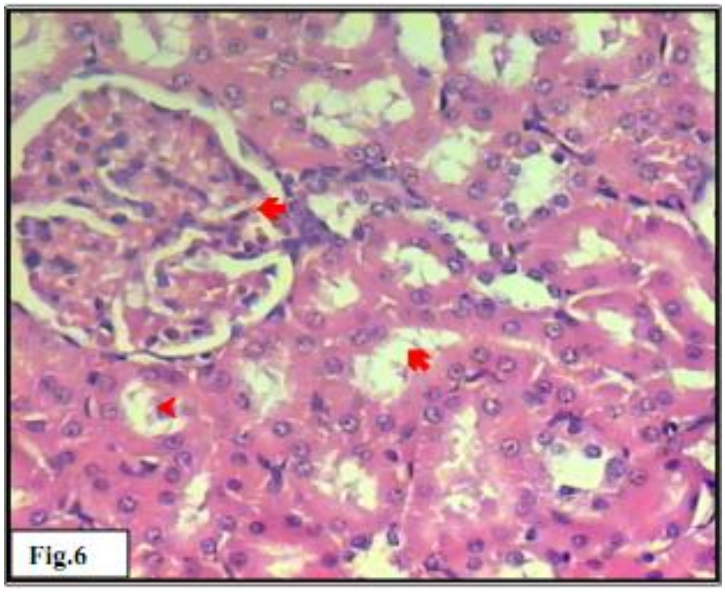

FIGURE 6: A section of the kidney of control mice showing a renal corpuscle (arrowhead), proximal convoluted tubules (←), and distal convoluted tubules (↖) (H & E X 400).

On the other hand, kidney of the treated mice showed in dosage 0.8 mg /kg body weight for 15 and 30 day after the dosage, large area hemorrhage and dilation in the renal tubules (Figure 7a) and congested blood vessels that associated with thickened wall and aggregation of inflammation cells (Figure 7b). There was a significant alteration in the histoarchitecture of the kidneys in dosage 1.6 mg /kg body weight, especially in the end of the experiment. The section of the kidneys after 15 days of insecticide exposure showed degeneration changes (Figure 8a). The nephritic changes continued as the experiment progressed, with marked congestion, collecting duct degeneration and sloughing of epithelial cells becoming evident on day 30 of treatment (Figure8b).histological changes did not notice clear in kidney tissue at the dosage of 0.2 and 0.4 mg / kg of body weight and for the two periods of 15 and 30 days of dosing respectively.

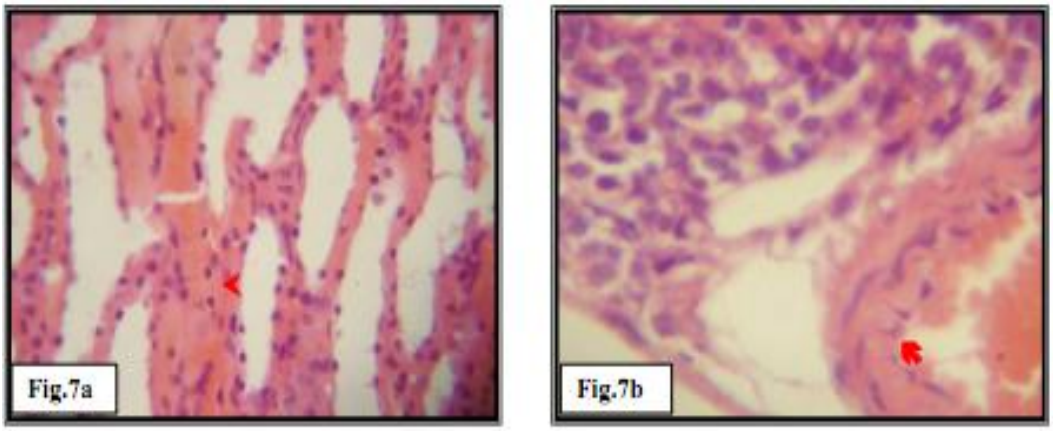

FIGURE 7: A) A section of kidney of the treated mice in dosage o.8 mg/kg body weight for 15 day after dosage showing large area hemorrhage and dilation in the renal tubules (arrowhead). b) A section of kidney of the treated mice in dosage o.8 mg/kg body weight for 30 day after dosage showing congested blood vessels that associated with thickened wall (↖) and aggregation of inflammation cells(←) (H&E X400).

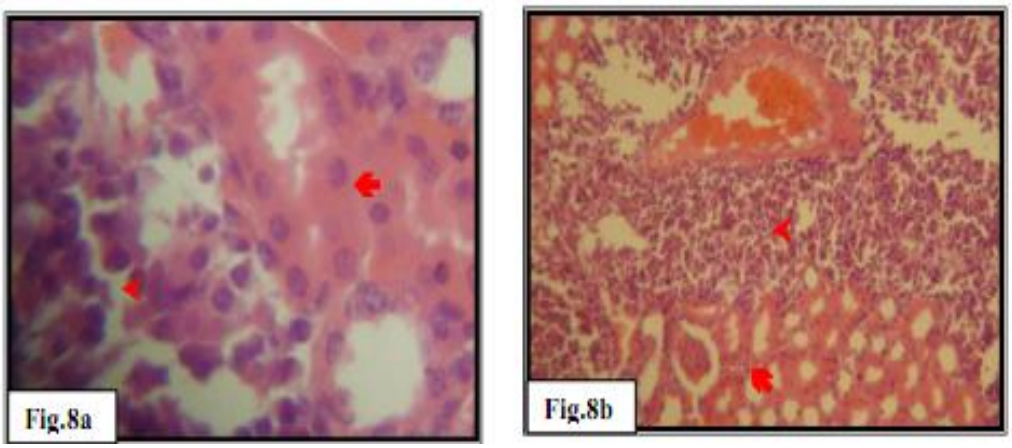

FIGURE 8: A) A section of kidney of the treated mice in dosage 1.6 mg/kg body weight for 15 day after dosage showing congestion (🡐) and degeneration (arrowhead). b) A section of kidney of the treated mice in dosage 1.6 mg/kg body weight for 30 day after dosage showing congestion and hemorrhage areas in the interstitial space between the tubules and glomeruli (🡔) and aggregation of inflammation cells (arrowhead) (H&E X400).

DISCUSSION

The present study revealed that treated mice by this insecticide showed different pathological lesions in the liver tissue. Microscopic changes in the liver with large areas of hydropic degeneration, large areas of necrosis and congested sinusoidal spaces after the 15th and 30 days of dosage. The study suggested that toxic responses occur relatively frequently in the liver compared with other organs, mainly because the liver is a predominant organ for the metabolism, and also is the center detoxifying any foreign compounds entering the body. So, it uniquely exposed to a wide variety of exogenous and endogenous products. These include environmental toxins and chemicals present in food or drinking (Goyal *et al.,* 2010; Marrs, 2012). These results are in accordance with histopathological lesions observed in livers of male albino rats exposed to thiamethoxam (Shalaby *et al*., 2010; EPA, 2002). The histopathological lesions obtained from the kidney were included hemorrhage areas in the interstitial space between the tubules and glomeruli. Also, congested blood vessels the associated with thickened wall and aggregation of inflammation cells was observed in kidney sections of some treated mice.

Kidneys are an important excretory organ, which, along with the liver, also accumulate toxicants. Chemically induced injury to the kidneys is reported to occur as a result of the direct effect of Actara or a metabolite on renal cells, or occurs indirectly by alteration in renal hemodynamics, or by a combination of both. The observed histopathological changes in the kidney in the present study clearly indicated that repeated oral exposure to Actara has a marked adverse effect on the functioning of kidneys in mice. This finding was similar to the report of EPA (2011) and Shridhar (2010) in other animals. In conclusion**,** generally, our results suggest that Actara insecticide may cause impairment of the histopathological parameters in mice. So, these effects may influence the use of this insecticide against pests attack vegetables in fruit stage.

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