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The Pathological Effect of Cypermethrin on Domestic Pigeons (*Columba livia gaddi*) at Basrah City/Southern Iraq

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Abstract: A two months of toxicologic pathology study of cypermethrin in domestic pigeons by oral intubation were done. The study was done at three dosage levels as: low (0.25 ml), intermediate (0.50 ml) and high (0.75 ml) with untreated control as fourth group. Histopathological changes found that in the heart areas of vacuolation of myocardial muscle cells were noticed in all treated groups, some with foci of fat cells between myocardial muscles cells. While, in the liver there were many pathological changes like; a minimal diffuse vacuolation of hepatocytes and several foci of inflammatory cells mainly mononuclear, other with periportal fibrosis and septal fibrosis and moderate diffuse vacuolation of hepatocytes, also, granuloma with multinucleate giant cells and foci of mononuclear cells one with microgranuloma and a suppurative granuloma and foci of mononuclear cells. In spleen's pigeons found atrophic lymphoid tissue, some with perifollicular fibrosis, other with a minimal atrophic of white pulp lymphoid tissue. In pancreas a vacuolation of islets of langerhans were noticed in all treated groups. In lungs with low, intermediate and high dose the pathological changes were found an emphysema and congestion, some with foamy alveolar macrophages. In low dose of cypermethrin there was no pathological changes in kidney, but in intermediate and high dose a dilated cortical tubules were found. In brain at all treated groups found a normal without any pathological changes. The sciatic nerve in pigeons with low and intermediate dose of cypermethrin found an occasional degenerate vacuolated nerve fibers, prominent shwann cells. While, in high dose a several degenerate vacuolated nerve fibers were noticed. An occasional degenerate vacuolated nerve fibers were found in spinal cord of pigeons with low dose, while, in intermediate dose a few degenerate vacuolated nerve fibers. In high dose a numerous degenerate vacuolated nerve fibers were found.

Key words: Cypermethrin, pigeon, oral intubation, histopathological changes, vacuolation

INTRODUCTION

Cypermethrin is the ISO approved common name for (RS)- α -cyano-3-phenoxybenzyl (1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate, it is a synthetic pyrethroid insecticide containing three chiral centres, giving a racemic mixture of eight isomers comprising four diastereoisomeric pairs. The cypermethrin are alpha-cyano- or type II pyrethroids (http://www.who.int/whopes/quality/en/Alphacypermethrin_eval_april_2006). It was first evaluated by the 1979 JMPR, when a temporary ADI was established and new toxicological data were evaluated at the 1981 JMPR and an ADI of 0-0.05 mg/kg bw per day was established (www.Cypermethrins.com, 2011). Cypermethrin was reviewed by the present Meeting within the periodic review programme of the CCPR; this review included alpha-cypermethrin and zeta-cypermethrin, which had not previously been considered by the JMPR (www.Cypermethrins.com, 2011). It was commonly used to control various pests, including moth pests of cotton, fruit and vegetable crops (Meister, 1992). It is also used for crack, crevice and spot treatment to control insect pests in stores, warehouses, industrial buildings, houses and apartments, greenhouses, laboratories, ships, railcars, buses, trucks and aircrafts. It may also

be used in nonfood areas in schools, nursing homes, hospitals, restaurants, hotels and food processing plants (Anonymous, 1989). Moreover, it was being used in veterinary practice against ectoparasites, so, cypermethrin is toxic not only for insects but also for mammals (He, 2000; Barlow *et al.*, 2001). Seth *et al.* (2000); Brown (2005) and Valez *et al.* (2008) found that the main target organ of cypermethrin was the nervous system, it's acts on the sodium channel in the nerve membrane by delaying the closing for several seconds. So that, the rapid absorption, metabolism, wide distribution and slow elimination make scientists classify this material as class II in toxicity (WHO, 1997; Reigart, 1999; Beyerbach, 2000). And Inducing many effects on hematological, biochemical, dermatological, muscular, urinary, central and peripheral nervous system and digestive system (Tample and Smith, 1996). In the world there are many studies about cypermethrin on different animals, Ullah *et al.* (2006) study the fetotoxic effects of cypermethrin in female rabbits with two different doses and founded that there was a significant difference in the numbers of fetuses which mean the early embryonic death and post implantation loses at the high dose and a microscopic changes found in the ovaries and uteri of animals treated with it.

While, Sangha *et al.* (2011) found that a Significant changes in body weight and various organ weights due to cypermethrin were observed along with disruption of estrous cycle in rats. Manna *et al.* (2004) reported a decreased in activities of Catalase (CAT), Superoxide Dismutase (SOD) and glycogen in the liver and increased the serum Aminotransaminases (AST, ALT), Alkaline Phosphatase (ALP) and lactate dehydrogenase (LDH) activities and blood glucose level with some cytotoxic effect on the lungs, liver, stomach, intestine, testes and cerebellum in rats.

Sayim *et al.* (2005) focused that there were a significant increases between initial body weights and final body weights of rats which treated with cypermethrin and a significant decrease in brain/body weight ratio of the animals of all treated groups. But no statistical difference between control group and all experimental groups for brain acetylcholinesterase and blood cholinesterase enzyme activities, while, brain acetylcholinesterase activities were increased in rats treated with cypermethrin and histologically, some deformation areas due to ischemia and pyknosis of the cytoplasm of the neurons were observed in brain tissues of rats treated with all doses of cypermethrin. Ahmad *et al.* (2009) showed a significant increase in Mean Corpuscular Volume (MCV) and significant decrease in TEC, Hb concentration and Mean Corpuscular Hb Concentration (MCHC) were founded in male rabbits treated with cypermethrin, while decrease in these parameters in female rabbits and concluded that macrocytic hypochromic anemia was induced in the male rabbits due to cypermethrin treatment, but not in the females.

Although enough information is available about cypermethrin toxicity from different regions of the world and for different animals, yet little work has been accomplished on cypermethrin toxicity in wild and domestic pigeon. Therefore, to protect the birds owner and their animals from the toxic effects of cypermethrin, this project was designed with the objectives to investigate clinical signs/symptoms of cypermethrin toxicity and gross and histopathological lesions related in pigeons as a model at Basrah city/southern Iraq.

MATERIALS AND METHODS

Experimental model: A total of (40) adult domestic pigeons were purchased from different owners in local market in Basrah city and reared in a clean cages (200 x 100 x 80 cm) in poultry unite/college of veterinary medicine/Basrah university, all pigeons were acclimatized for 10 days before start the experiment.

Chemicals: Cypermethrin from manufacturing chemicals Ltd. England were used under this study, then, it was further diluted in distilled water to obtain the desired concentration. The solution was prepared and used

immediately, by oral gavage using disposable syringe after removing the needle, the doses of cypermethrin were determine by testing the compound on few pigeons and the maximum toxic dose which was used according to the active ingredients of the substance.

Treatments: To study the toxologic pathology of cypermethrin on domestic pigeons a total of forty birds were randomly divided into four groups (10 pigeons each group); group one as low dose of 0.25 mg/day cypermethrin, group two as intermediate dose of 0.5 mg/day cypermethrin, group three as high dose 0.75 mg/day cypermethrin, fourth group was given 1 ml distilled water as untreated control group. The experiment was done for two months, after the end of the experiment all pigeons were killed by cervical dislocation, selected visceral organs were fixed in 10% neutral buffered formalin for further histopathological study.

Histopathological examinations: Four μ m thick paraffin sections were prepared as follows: a visceral organs tissues of liver, kidney, spleen, lung, pancreas, heart, brain, spinal cord and sciatic nerve from each pigeon were fixed in 10% neutral buffered formalin, then samples were cut and paraffin blocks were made, slide were cut and stained with Haematoxyline-Eosin (HE), selected histopathological changes were photographed from treatment related histopathological changes in comparison to untreated controls, according to the method of Luna (1968).

RESULTS

After two months of the experiment, a post mortem were examine and founded that no gross lesions in any organ, but spleen appeared as a dark black in color in pigeons with high dose of cypermethrin.

The histopathological effects of cypermethrin on different organs of domestic pigeons were founded as follows:

Heart: Areas of vacuolation of myocardial muscle cells were founded in all treated groups (Fig. 1, 2, 3), with foci of fat cells between myocardial muscles cells those were founded in intermediate and high dose(Fig. 2, 3) as compared with control (Fig. 4).

Liver: In liver there were many pathological changes that were noticed, like; a minimal diffuse vacuolation of hepatocytes and several foci of inflammatory cells mainly mononuclear, other with periportal fibrosis and septal fibrosis in low dose of cypermethrin (Fig. 5), while, in intermediate dose a moderate diffuse vacuolation of hepatocytes, also, granuloma with multinucleate giant cells and foci of mononuclear cells one with microgranuloma (Fig. 6). A suppurative

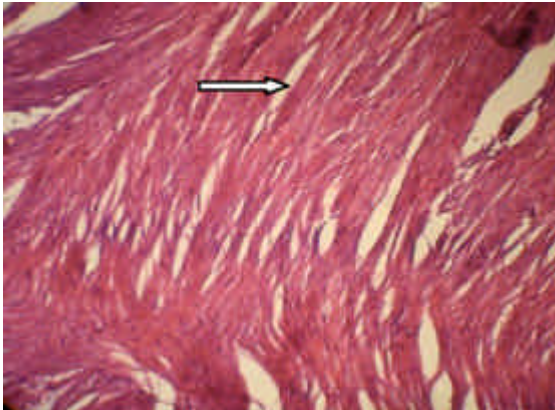


Fig. 1: Heart of pigeon with low dose of cypermethrin, vacuolation of myocardial muscle cells. H&E (500X)

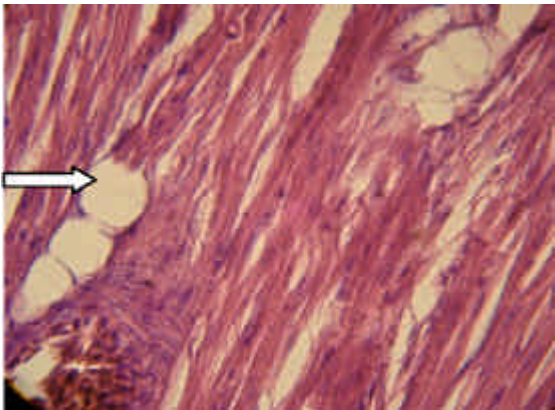


Fig. 2: Heart of pigeon with intermediate dose of cypermethrin, vacuolation of myocardial muscle cells and infiltration of fat cells between myocardial muscle cells. H&E (500X)

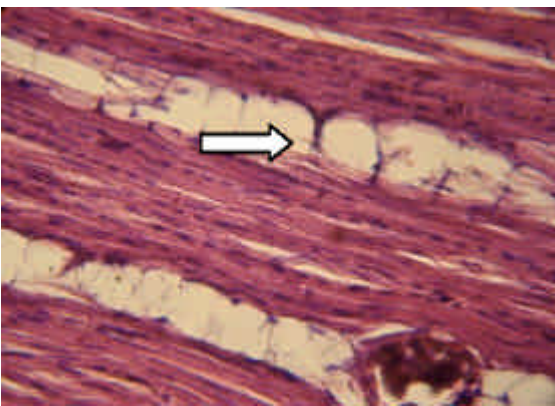


Fig. 3: Heart of pigeon with high dose of cypermethrin, vacuolation of myocardial muscle cells and foci of fat like cells between myocardial muscle cells. H&E (500X)

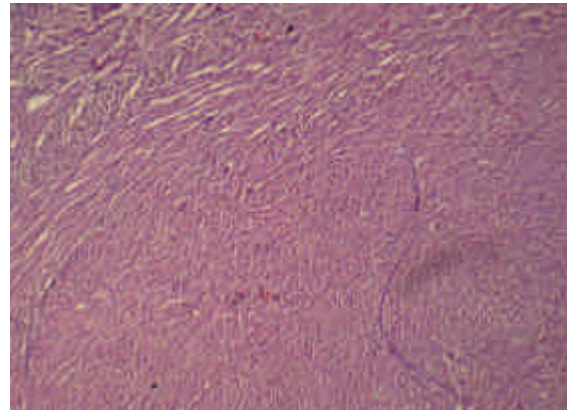


Fig. 4: Heart of pigeon as control group. H&E (125X)

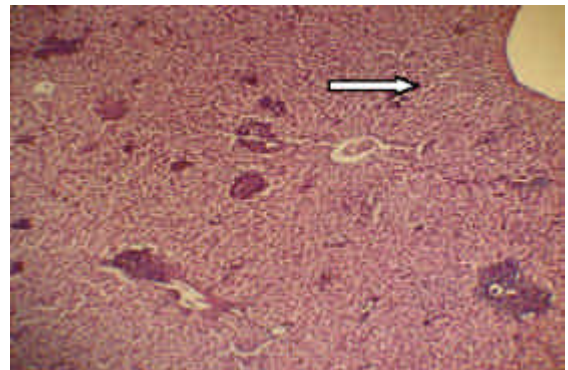


Fig. 5: Liver of pigeon with low dose of cypermethrin with minimal diffuse vacuolation of hepatocytes and several foci of inflammatory cells. H&E (125X)

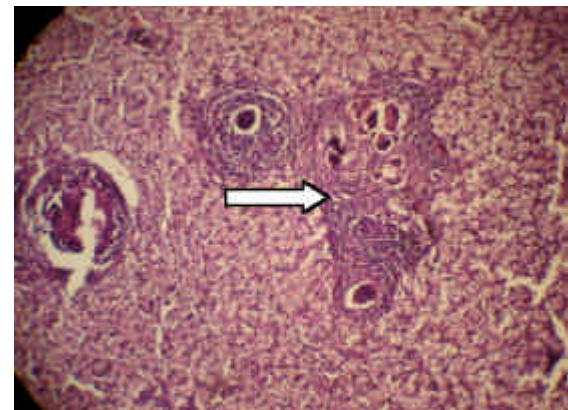


Fig. 6: Liver of pigeon with intermediate dose of cypermethrin a granuloma with multinucleate giant cells. H&E (500X)

granuloma and foci of mononuclear cells, others aggregate of lymphocytes were noticed in liver with high dose of cypermethrin (Fig. 7) as compared with control (Fig. 8).

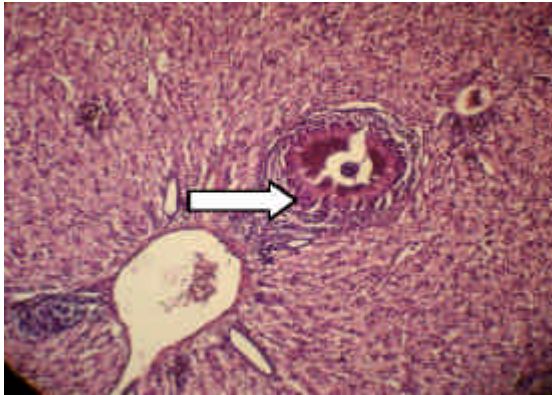


Fig. 7: Liver of pigeon with high dose of cypermethrin with suppurative granuloma and foci of mononuclear cells. H&E (500X)

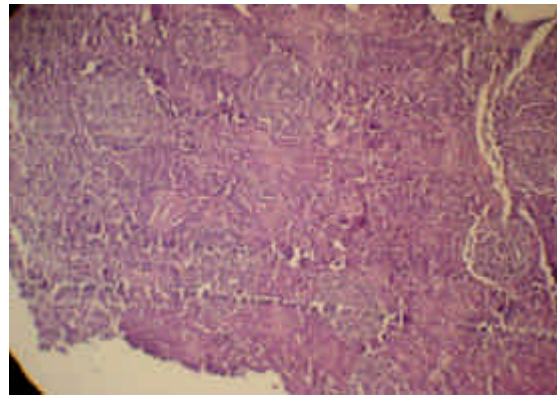


Fig. 10: Spleen of pigeon with low dose of cypermethrin, atrophy of lymphoid tissue. H&E (125X)

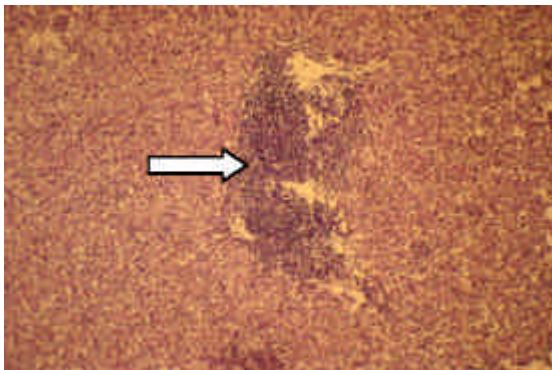


Fig. 8: Liver of pigeon with high dose of cypermethrin with area of aggregate of lymphocytes. H&E (125X)

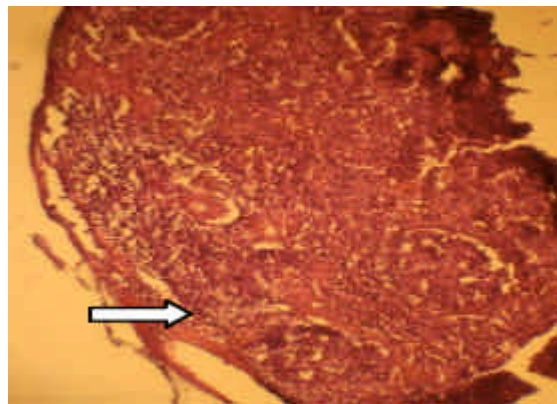


Fig. 11: Spleen of pigeon with intermediate dose of cypermethrin, atrophy of lymphoid tissue and perfollicular fibrosis. H&E (500X)

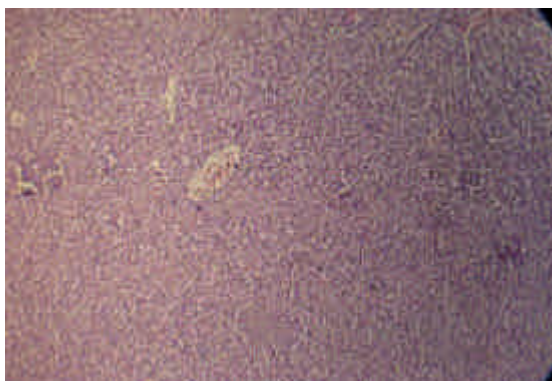


Fig. 9: Liver, control. H&E (125X)

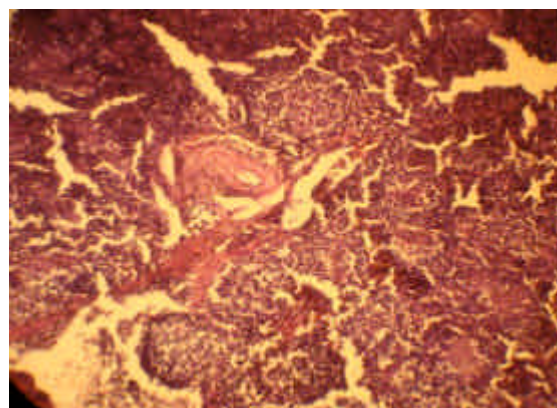


Fig. 12: Spleen of pigeon with high dose of cypermethrin, minimal atrophy of white pulp lymphoid tissue. H&E, (500X)

Spleen: In pigeons with low dose of cypermethrin the spleen found with atrophic lymphoid tissue (Fig. 10), while, in intermediate dose atrophic lymphoid tissue and perfollicular fibrosis (Fig. 11). In high dose a minimal atrophy of white pulp lymphoid tissue (Fig. 12). As compared with control (Fig. 13).

Pancreas: In all treated groups under this study a vacuolation of islets of Langerhans were noticed (Fig. 14).

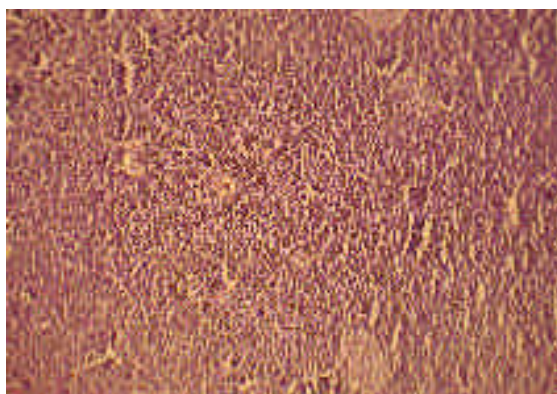


Fig. 13: Spleen, control. H&E (125X)

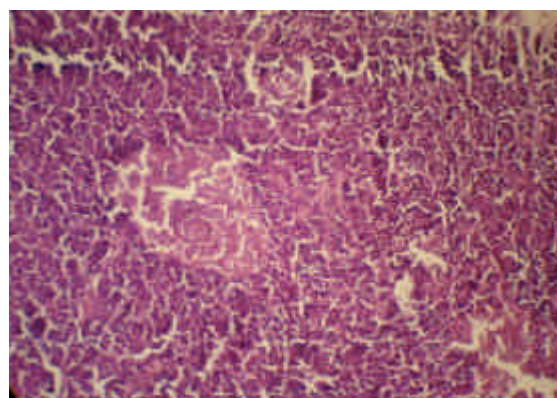


Fig. 14: Pancreas of pigeon treated with cypermethrin showed a vacuolation cells of the islets of Langerhans. H&E (500X)

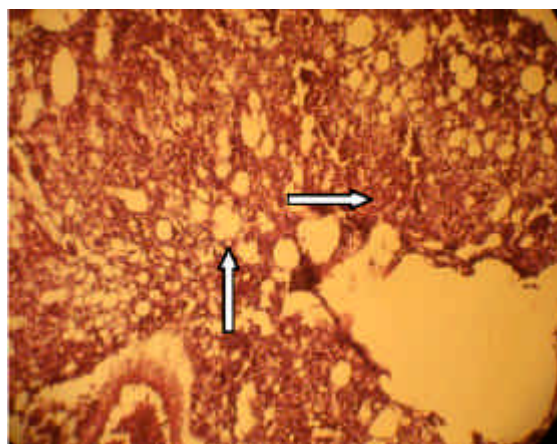


Fig. 15: Lung of pigeon with low dose of cypermethrin an congestion and emphysema. H&E (125X)

Lung: In lungs with low, intermediate and high dose of cypermethrin the pathological changes were founded an emphysema and congestion, some with foamy alveolar macrophages (Fig. 15, 16), as compared with control group (Fig. 17).

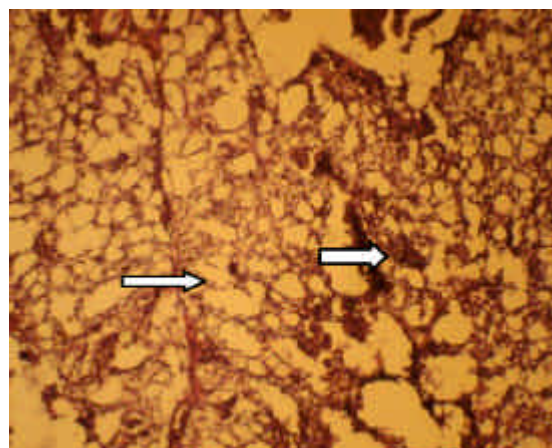


Fig. 16: Lung of pigeon with intermediate dose of cypermethrin with congestion and emphysema, areas of foamy alveolar macrophages. H&E (500X)

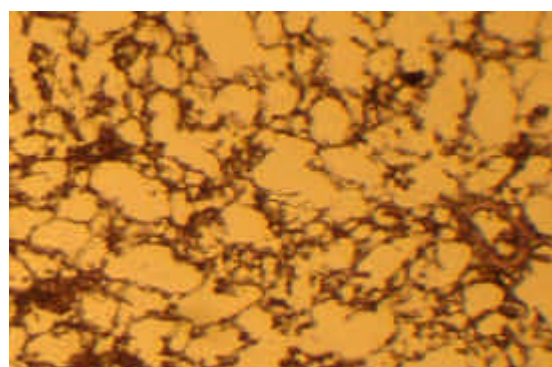


Fig. 17: Lung, control group. H&E (125X)

Kidney: In low dose of cypermethrin there was no pathological changes, but in intermediate and high dose a dilated cortical tubules were founded (Fig. 18, 19), as compared with control group (Fig. 20).

Brain: All the treated groups founded a normal brain without any pathological changes (Fig. 21).

Sciatic nerve: The sciatic nerve in pigeons with low, intermediate and high dose of cypermethrin founded with occasional degenerate vacuolated nerve fibers, prominent shwann cells (Fig. 22, 23, 24). As compared with control (Fig. 25).

Spinal cord: An occasional degenerate vacuolated nerve fibers were founded in pigeons with low dose of cypermethrin (Fig. 26), while, in intermediate dose a few degenerate vacuolated nerve fibers (Fig. 27). In high dose a numerous degenerate vacuolated nerve fibers were founded (Fig. 28). As compared with control group (Fig. 29).

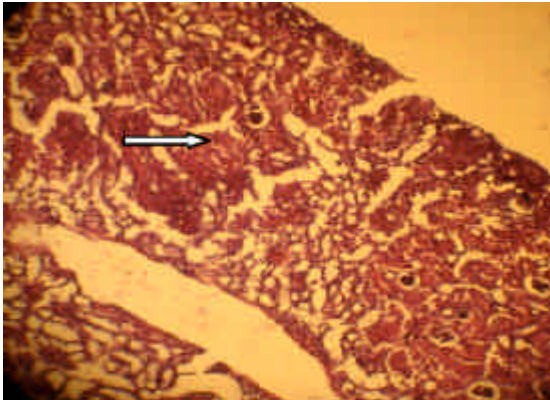


Fig. 18: Kidney of pigeon with intermediate dose of cypermethrin with dilated cortical tubules. E&H (125X)

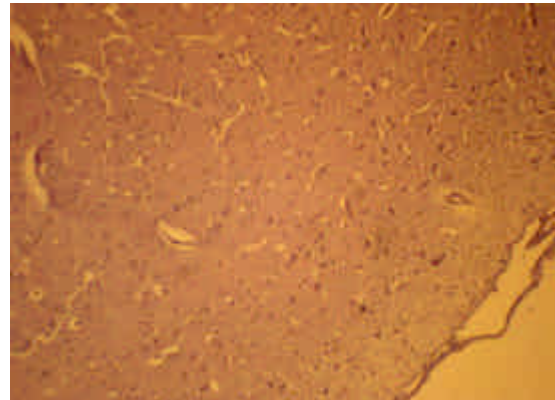


Fig. 21: Brain of treated groups with cypermethrin appeared as normal. H&E (125X)

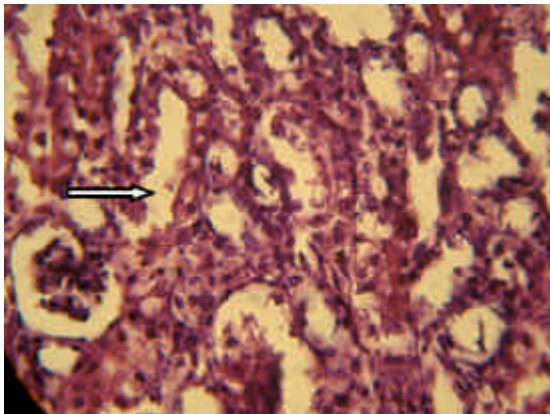


Fig. 19: Kidney of pigeon with high dose of cypermethrin showed a dilated cortical tubules. E&H (500X)

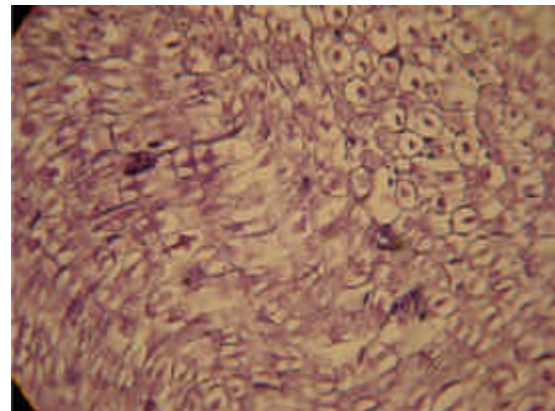


Fig. 22: Sciatic nerve of pigeon with low dose of cypermethrin with occasional degenerate vacuolated nerve fibers. H&E (500X)

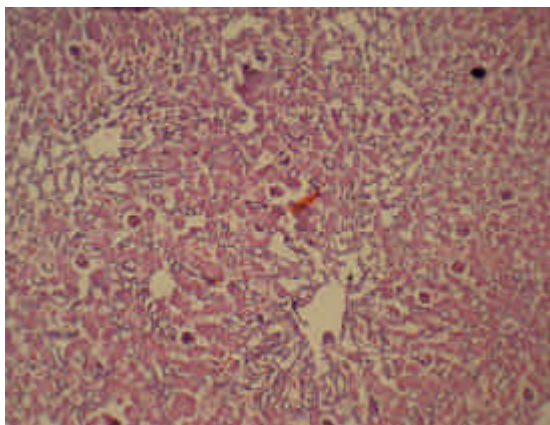


Fig. 20: Kidney, control. H&E (125X)

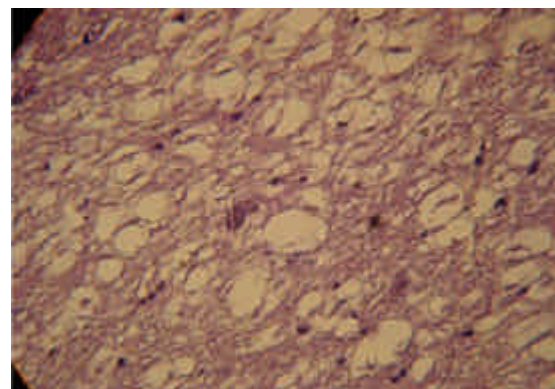


Fig. 23: Sciatic nerve of pigeon with intermediate dose of cypermethrin with occasional degenerate vacuolated nerve fibers, prominent schwann cells. H&E (500X)

DISCUSSION

Cypermethrin is a synthetic pyrethroid insecticide which founded in the flower head of *Chrysanthemum* spp.

(WHO, 1989; Cox, 1996) and it was used for more than 40 years and accounts for 25% of the worldwide

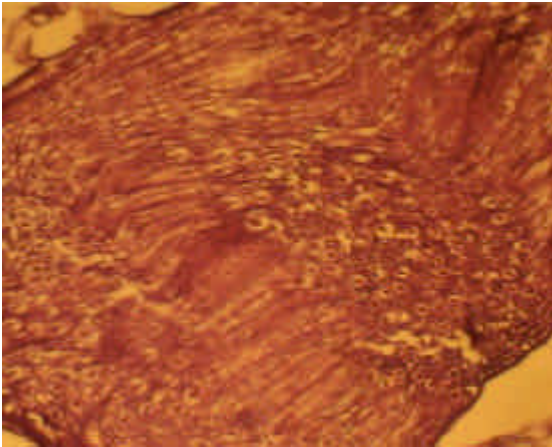


Fig. 24: Sciatic nerve of pigeon with high dose of cypermethrin a several degenerate vacuolated nerve fibers. H&E (125X)

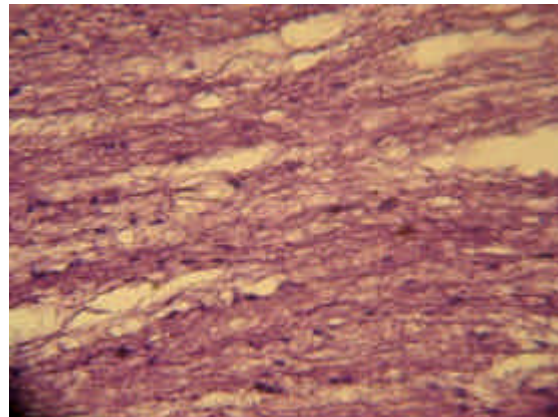


Fig. 27: Spinal cord of pigeon with intermediate dose of cypermethrin a few degenerate vacuolated nerve fibers. H&E (500X)

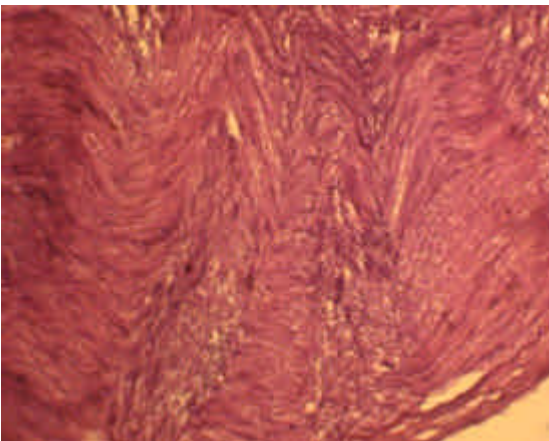


Fig. 25: Sciatic nerve of pigeon, control. H&E (125X)

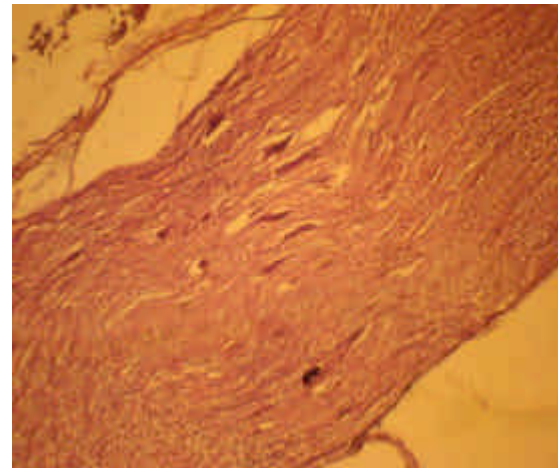


Fig. 28: Spinal cord of pigeon with high dose of cypermethrin a numerous degenerate vacuolated nerve fibers. H&E (125X)

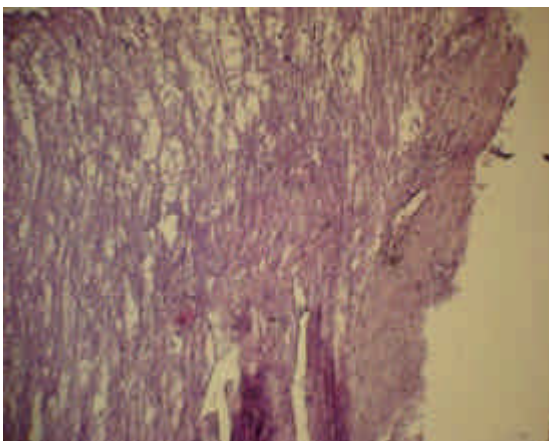


Fig. 26: Spinal cord of pigeon of low dose of cypermethrin an occasional degenerate vacuolated nerve fibers. H&E (125X)

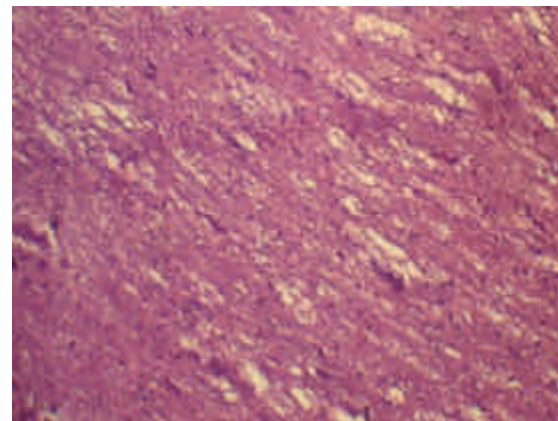


Fig. 29: Spinal cord of pigeon with control group. H&E (125X)

insecticide market (Khan *et al.*, 2003). It was a wide using in veterinary medicine, like; dipping, spraying, pour-on and spot-on (Harold *et al.*, 2003; Sudakin, 2006), furthermore, it has a high insecticide activity with a low avian and mammalian toxicity and important in agricultural uses and plant protection (Vijerberg and Van Berken, 1990; Baker *et al.*, 2007).

The current study didn't find any gross lesions post mortem at the end of the study, but spleen with a dark black color in high dose group. This may be related to congestion as a results of the stress factors, this result agree with (Al-Autaish, 2010). Yousef *et al.* (1998) reported that the dark color of spleen in sheep exposure to cypermethrin due to excessive damage of erythrocytes as a results to the aromatic amine toxicity and sequestration of the damaged erythrocytes in splenic sinusoids.

Heart with vacuolation and foci of fat cells may be as a result of toxic effects of cypermethrin. The pathological changes which found in the liver make sure that cypermethrin a high toxic on liver and enzymes and may be cypermethrin metabolized in the liver by hydrolytic ester cleavage and oxidative pathway by the microsomal enzyme system. Khan *et al.* (2003) reported that pathological changes in liver of animals that exposure to cypermethrin influence of toxic compound in the digestive tract.

The pathological changes in spleen and pancreas of pigeons with all treated groups revealed that cypermethrin was a toxic component in birds which is disagree with (www.Cypermethrins.com, 2011; http://www.who.int/whopes/quality/en/Alphacypermethrin_eval_april.2006).

The first record of pathological effect of cypermethrin in lung of pigeons under this study was foamy alveolar, all the researches in different animals reported a congestion, hypertrophy and emphysema. This may be due to an toxic component cause a damaged to the alveoli, as it's noticed by Khan *et al.* (2003) and Al-Autaish (2010). While, the pathological changes in kidney make sure that cypermethrin a hyperactivity and detoxication and the dilated cortical tubules due to the filling with protein casts, as the same with (Khan *et al.*, 2003).

The pathological finding in the nervous organs (brain, sciatic nerve, spinal cord) under this study revealed that brain was normal and it's may be that a short term exposure of pigeons to the cypermethrin didn't reach to the brain and make a changes, while, in the sciatic nerve and spinal cord the changes may due to neurotoxic effect of cypermethrin. Iwanika and Borzecki (2008) noticed that a very rapid distribution in the nervous system within five minutes after intravenous administration in rats. While, Yousef *et al.* (1998) reported that a swelling myelin sheath and breaking of some axons of sciatic nerves as a result for cypermethrin effects on barks sheep.

Cypermethrin is practically non-toxic to birds, but is very highly toxic to fish and aquatic invertebrates. This is mainly because it is metabolized and eliminated significantly more slowly by fish than by mammals or birds and classified as a Schedule poison in the Standard for the Uniform Scheduling of Drugs and Poisons (Tasmania Issue Publishing, 2002). Although the mechanism of toxicity of pyrethroids has not been fully explored, various opinions have been put forward. CY can induce oxidative stress in blood cells (Kale *et al.*, 1999) or may accrue in cell membranes and disturb structure of membrane (Michelangeli *et al.*, 1990).

Cypermethrin, alpha and zeta cause neurotoxicity in mammals and insects by causing a long-lasting prolongation of the normally transient increase in sodium permeability of nerve membrane channels during excitation. Salivation and tremors that progress to clonic-tonic convulsions (http://www.who.int/whopes/quality/en/Alphacypermethrin_eval_april.2006). In conclusion the cypermethrin made many pathological effects on different organs of domestic pigeons, so, the owners of birds must be draw attention and make sure about feeding materials which didn't exposure to this toxic component.

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