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Effects of Emamectin Benzoate on Hematological, Biochemical and Histopathological Parameters in Male Pigeon (*Columba livia domestica*)

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ABSTRACT

The pesticides tend to become concentrated as they move up the food chain. They accumulate in the organism body (target and non-target organisms) which contained them. The pesticides don't kill the individual birds often but do affect their bodies so that they lay eggs with very thin shells often these thin-shelled eggs break or the birds are unable to reproduce. Pesticides also affect bird's physiological functions. The natural conservation committee and the Royal Society for bird protection suggested that the indirect effect of pesticides was a major cause of the decline of bird species. Emamectin benzoate (EB) (4″-deoxy- 4″-epi-methyl-amino benzoate salt of avermectin B, (abamectin), is a widely used broad-spectrum insecticide. Despite the increasing use of Emamectin benzoate in Yemen, there is no complete information on the toxic effects of this insecticide in birds. Pigeons usually feed on the water and seeds that may be contaminated by the insecticide (Emamectin benzoate), meanwhile, their meat is greatly required as food for people. Therefore, the purpose of this study was to investigate the effects of different oral dosage toxicity of Emamectin benzoate in male pigeons for 45 successive days. The bird employed in the present study is the pigeon (*Columba livia domestica*), weighing between 200–320 g . Birds were classified into four groups each consists of 5 animals as follow: Group (I) control group were administrated with normal diet and water, Group (II): Pigeons were administrated daily with (2 mg/kg body weight) Emamectin benzoate, Group (III): was administrated daily with (4 mg/kg/bw) Emamectin benzoate, Group (IV): were administrated daily with (8 mg/kg/bw) Emamectin benzoate. All doses were dissolved with water for 45 consecutive days. The result showed a significant decrease in the body weight at a dose (4 and 8 mg/kg/bw) of EB and there was a significant decrease in all the investigated hematological parameters with dose (4 and 8 mg/kg/bw) EB. Oral administration of EB to a male pigeon for 45 days resulted in significant inhibition of total protein and albumin while a significant increase in serum urea and creatinine compared to the control group. It may be suggested that 2 mg/kg/day for Emamectin benzoate produced no desirable effects, therefore these doses may be considered as No Observed Effect Level (NOEL) for insecticides to male pigeons.

INTRODUCTION

The toxicity of pesticides to birds has long been a matter of concern to conservationists and is a factor taken into consideration by regulatory authorities when making environmental risk assessments for new chemicals. Where the toxicity of a pesticide is found to be substantially and consistently different between birds and mammals it is of interest to establish the toxicokinetic and/or toxicodynamic factors which are responsible. A number of pesticides have been shown to cause the deaths of birds even when used in an approved fashion in agriculture and horticulture. Thus, persistent organochlorine insecticides have been implicated in the deaths of grain-eating and predatory birds[1], and non-persistent insecticides of high acute toxicity such as carbophenothion and aldicarb have caused deaths of Anser geese and gulls respectively[2,3]. In the United Kingdom, the Annual Report of the Wildlife Incident Scheme (M.A.F.F.) draws attention to a considerable number of bird casualties on agricultural land attributable to a range of non-persistent pesticides (usual insecticides) each year. Until now, attention has been focused upon the lethal effects of single chemicals. However, birds are exposed to combinations of chemicals under field conditions and there has been growing concern about possible toxic effects which may be the consequence of interactions between pesticides. This study dialed with the toxicological consequences of the induction or inhibition of enzymes involved in the metabolism of pesticides[4].

Pesticides primarily affect the nervous system of the exposed organisms by inhibiting acetyl cholinesterase (AChE) and raising acetylcholine levels in the cholinergic synapse. Besides cholinergic effects, pesticides induce oxidative stress[5,6], affect metabolic pathways[7], and cause multiple organ dysfunctions such as hypoxia and inadequate tissue perfusion of the liver and heart[8]. In the liver, they cause ultrastructure, biochemical, metabolic and mitochondrial damage, evidenced by changes in hepatic biomarkers such as serum aminotransferase and direct and indirect bilirubin[9,10]. Their mechanisms of action on the liver and metabolism have not yet been fully clarified and finding an effective therapy against pesticides remains a major challenge.

Emamectin benzoate (EB) is the 4″-deoxy- 4″-epi-methyl-amino benzoate salt of avermectin B, (abamectin), which is similar structurally to natural fermentation products of soil actinomycete, *Streptomyces avermitlis*. Emamectin benzoate is currently being developed for the control of lepidopterous pests on a variety of vegetable crops worldwide with a very low application[11]. It is also extremely effective for treating and controlling sea lice infestations

on Atlantic salmon, Salmo solar L[12]. The mechanism of action involves the blocking of γ-amino butyric acid-stimulated (GABA) chloride channels and open non-neurotransmitter-gated chloride causing an ion imbalance in the nervous system, resulting in paralysis and death[13]. In 1997, Wise *et al* [14] conducted laboratory tests on pregnant female Sprague-Dawley rats which orally gavaged EB once daily. The results demonstrated that the high-dose of Emamectin benzoate (3.5/2.5 mg/kg/bw) exposure during gestation and lactation to rats produced evidence of neurotoxicity in the F1 offspring and the calculated no observed effect level (NOEL) for developmental neurotoxicity of EB was determined to be 0.6 mg/kg/bw/day. At the same time, groups of rats (20/sex/dose) were given Emamectin (as hydrochloride salt) at 0, 0.5, 2.5 or 12.5/ 8/5 mg/kg bw/day in their diet for 14 weeks. The highest dose was reduced to 8 mg/kg bw/day at the 3rd week, and subsequently to 5 mg/kg bw/day at the 9th week. Significant reductions in body weight and food consumption were observed in animals receiving 12.5/ 8/5 mg/kg bw/day. Decreased serum glucose concentration and a slight increase in blood urea nitrogen were detected at all sampling times following treatment with 12.5/ 8/5 mg/kg bw/day. Neuronal cytoplasmic vacuolation and degeneration were also noted. The NOEL was 2.5 mg/kg bw/day based on neurotoxicity, weight loss and decreased food consumption in rats who received high doses[15].

Emamectin benzoate insecticides are currently used in great amounts in Yemen and abroad, but this can rise a problem when the possible risks of occupational and environmental contamination are taken into account. Since EB is now being considered to replace other existing pesticides, therefore the relative risk and benefits of this insecticide must be compared to the existing pesticide. Although the data about the action of EB on GABA receptors in insects are clear, there are a few works that demonstrate possible in vivo effects of pesticides on the mammalian biological system. Despite the increasing use of Emamectin benzoate in Yemen, there is no complete information on the toxic effects of this insecticide in birds. Pigeons usually feed on water and seeds that may be contaminated by the insecticide (Emamectin benzoate). For these reasons, the present study has been carried out to evaluate 45 days of oral toxicity of EB on male pigeons by studying hematological, biochemical and histopathological parameters.

MATERIAL AND METHODS

Pesticides used:

Emamectin benzoate was purchased from the local market of Ibb city, Yemen.

Experimental animal:

The male bird employed in the present study is the Pigeon (*Columba livia domestica*) which belongs to order columbiformes, weighing between 200-320g. Experimental birds were purchased from the local market of Ibb city, Yemen. They were healthy, active and free from any abnormalities. Birds were kept for one week under normal conditions of feeding with free access to water before experiments to assure their acclimatization.

Experimental design

Twenty male pigeons birds were randomly divided into four groups each consists of 5 animals as follow:

Group (I): control group was administrated with a normal diet and water daily for 45 days.

Group (II): Pigeons in this group were administrated daily with 2 mg/kg body weight Emamectin benzoate.

Group (III): Pigeons in this group were administrated daily with 4 mg/kg body weight Emamectin benzoate.

Group (IV): Pigeons in this group were administrated daily with 8 mg/kg body weight Emamectin benzoate. All doses were dissolved with water for 45 consecutive days.

Bodyweight and clinical signs of toxicity were recorded throughout the experiment. At the end of this experiment (45 days), blood samples were individually collected from each bird, immediately after slaughtering in dry clean centrifuge tubes and divided into two portions. The first portion was taken on 10% EDTA tubes (ethylenediaminetetraacetic acid) for hematological examination. The second portion was left to clot at room temperature for about 20 minutes and then centrifuged at 3000 r.p.m for 15 minutes; the supernatant serum samples were drowned in dry clean-capped tubes and kept in a deep freezer at –20 C^{o} until conducting the biochemical analysis.

Determination of Hematological Parameters

Hematological parameters [Red blood cell (RBC), white blood cell (WBC) counts, packed cell volume (PCV), hemoglobin concentration (Hb) and differential WBC counts] were determined using standard laboratory procedures as described by Cheesbrough[16]. Hematological indices including; mean corpuscular volume (MCV), mean corpuscular

hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated from the values of RBC count, PCV and Hb[16].

Determination of Biochemical analysis

Urea, Creatinine, Albumin, Total protein was estimated by using Roche diagnostic Kits (Germany) according to Tietz *et al*[17].

Histological analysis

Examination of renal histology was performed according to routine histology techniques. Briefly, after the animal was sacrificed, the kidney was put in normal saline and sectioned into small pieces. The sectioned tissue was then fixed in 10% formalin, dehydrated stepwise with increasing concentration of ethanol solution (50% to 100%) and embedded in paraffin. Using a microtome, tissue sections of 4 μm thickness were produced, fixed overnight on the slide, subsequently stained with Hematoxylin and Eosin (H&E) and were then observed under a light microscope (Olympus BX41, Japan).

Statistical analysis

Data were expressed as the mean values ± standard deviation (S.D.) for each measurement. The data were also analyzed by one-way analysis of variance (one-way ANOVA) using SPSS (version 20) the test is significant at α 5%.

RESULTS

Changes in body weight

Repeated oral administration of Emamectin benzoate at 2 mg/kg/bw did not produce any signs of toxicity and mortality during 45 days of exposure (Table 1). However, there was mortality and a significant decrease ($P \leq 0.05$) in the body weight of animals at a dose (8 mg/kg/bw day) of EB comparing to the control group. Repeated exposure to EB at doses 4 and 8 mg produces seizures, tremors, weight loss, or other types of apparent behavioral or physiological dysfunctions.

Table 1: Effect orally administered with different doses of Emamectin benzoate (EB) on body weight of male pigeon in grams (g).

Groups	Initial weight (g)	Final weight (g)
Control	244.00 ± 19.11	289.00 ± 139.12
EB (2 mg/kg)	282.00 ± 10.95[a]	269.00 ± 19.73[a]
EB (4 mg/kg)	255.40 ± 12.75[a]	234.80 ± 19.62[b]
EB (8 mg/kg)	249.20 ± 14.92[a]	231.40 ± 19.38[b]

Values across each column having the same superscript letter were not significantly different (P≤0.05). Each value represents the mean ± SD of 5 animals.

Effect of different doses of EB on Haematological Parameters

Present data showed that daily oral administration of male pigeons with Emamectin benzoate with doses 4 and 8 mg/kg/bw for 45 days significantly decreased (P≤0.05) all the investigated hematological parameters: RBCs, WBCs, Hb, MCV, HCT and Thrombocyte (Table 2).

Table 2: Effect of different doses of Emamectin benzoate (EB) on Haematological Parameters of male pigeons for 45 successive days

Parameters		Units	Control	EB (2 mg/kg)	EB (4 mg/kg)	EB (8 mg/kg)
Red Blood cells Count (R.B.C.)		$x10^6mm^3$	3.91 ± 0.52	2.60 ± 0.39*	1.73 ± 0.82*	1.57 ± 0.36*
Hemoglobin (Hb)		g. /dl	14.55 ± 0.27	13.96 ± 0.41	11.58±0.41*	8.89±0.31*
Packed Cell Volume (PCV)		%	37.43 ± 0.38	36.46 ± 0.96	34.97±0.74*	34.38±0.95*
Mean Corpuscular Volume (MCV)		fl	88.40 ± 0.35	70.28 ± 0.51*	67.32±0.32*	64.20±0.43*
MCH		pg	51.23 ± 1.05	48.61 ± 1.01*	28.87±0.75*	26.90 ± 0.89*
MCHC		g/l	30.24 ± 0.79	28.78 ± 0.64*	27.31±0.98*	25.96±0.28*
Platelets Count		g. /dl	12.44±0.28	10.74 ± 0.89*	6.92±0.73*	5.96 ± 0.40*
White Blood Cells (W.B.C.)		$x10^3mm^3$	20.63 ± 0.55	18.48 ± 0.64*	13.48±0.24*	12.12±0.65*
W.B.C. Differential count	**Heterophils**	%	45.00 ± 0.01	45.00 ± 0.01	43.00±0.01*	43.00 ± 0.01*
	Eosinophil's	%	2.00 ± 0.01	2.00 ± 0.01	4.00 ± 0.01*	3.00 ± 0.01
	Basophils	%	3.00 ± 0.01	3.00 ± 0.01	3.00 ± 0.01	3.00 ± 0.01
	Lymphocyte	%	50.00± 0.01	50.00 ± 0.01	47.00±0.01	48.00±0.01
	Monocyte	%	0.00 ± 0.01	0.00 ± 0.01	3.00±0.01	3.00±0.01

Each value represents the mean ± SD of 5 animals. * P≤0.05 compared with the normal control value.

Effect of different doses of EB on Biochemical Parameters

Serum urea and creatinine were determined as indicators of kidney functions since the increase in these components means that the kidney is the less active or abnormal case. Data in Table (3) showed a significant increase (P≤0.05) in serum urea and creatinine in male pigeons treated with a repeated dose of EB (4 and 8 mg/kg/bw) for 45 days when compared with the control group. EB caused significant inhibition in the levels of total protein and

albumin. Data showed significant increased (P≤0.05) levels of total cholesterol, HDL, LDL and triglycerides in pigeons treated with EB (Table 3).

Table 3: Effect of different doses of Emamectin benzoate (EB) on Biochemical Parameters of male pigeons for 45 successive days

Parameters	Units	Control	EB (2 mg/kg)	EB (4 mg/kg)	EB (8 mg/kg)
Urea	mg/dl	6.71 ± 0.08	10.60 ± 0.26*	12.32 ±0.43*	15.22 ± 0.34*
Creatinine	mg/dl	0.42 ± 0.02	0.94 ± 0.05*	1.62 ± 0.01*	1.66 ± 0.08*
Total protein	g/dl	5.34 ± 0.05	4.72 ± 0.04*	3.74 ± 0.35*	3.14 ± 0.46*
Albumin	g/dl	4.56 ± 0.08	3.58±0.32*	1.70±0.14*	1.40±0.12*
Triglyceride	mg/dl	273.64± 0.49	330.84 ± 1.62*	444.72±2.29*	460.66 ± 4.29*
Total cholesterol	mg/dl	185.64 ± 1.11	253.10 ± 1.83*	302.74±2.45*	325.60±2.38*
HDL	mg/dl	78.88±0.71	82.18 ± 1.10*	95.54±0.55*	97.78 ± 1.02*
LDL	mg/dl	67.90 ± 1.64	118.20 ± 1.43*	127.24±1.66*	172.18±1.34*

All value represents mean ± SD of 5 animals. * P≤0.05 compared with the normal control value.

Effect of different doses of EB on Kidney Histopathology

Microscopically examination of the kidneys of male pigeons from control illustrated the normal appearance of the glomerulus and renal tubules. However, kidneys of male pigeons administrated with EB (2 mg/kg/bw) showed mildly damaged glomeruli, dilatation of renal tubules. Histopathological changes were observed in the kidneys of male pigeons orally administrated with EB (4 and 8 mg/kg/bw) showed wide lumina, severely damaged glomeruli, interstitial vascular congestion and epithelial degeneration, dilatation of renal tubules, and highly vacuolation (Figure 1).

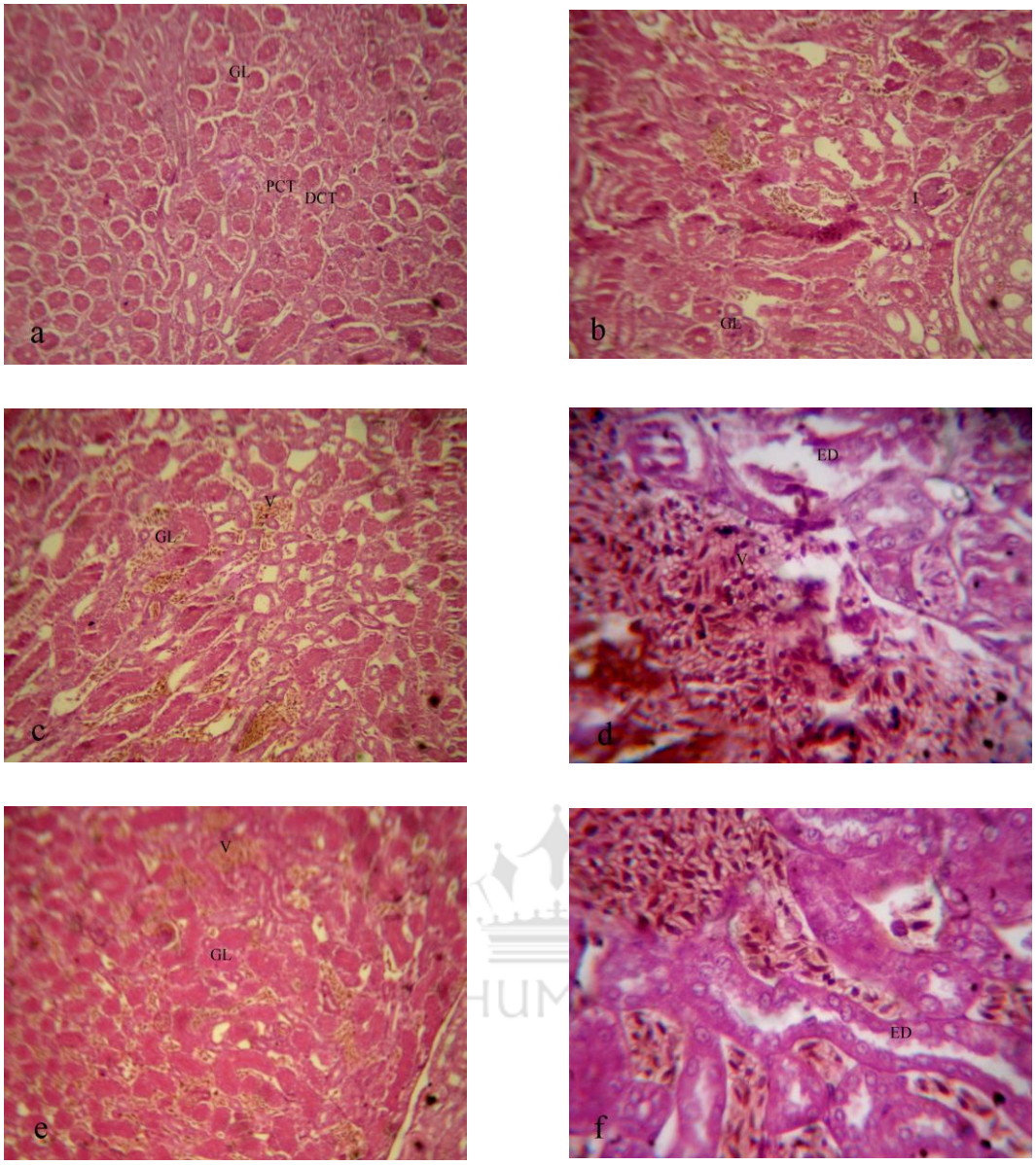

Figure 1: Histopathology of the kidney. a- Section of kidney from control group male pigeon showing the normal appearance of the glomerulus (GL) and renal tubules including proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) and interstitium (I). b- sections of the male pigeon administrated with EB 2 mg/kg/bw showed mildly damaged glomeruli (GL), tubular degeneration, and epithelial degeneration (I). c, d- sections of male pigeon administrated with EB 4 mg/kg/bw showed significant nephrotoxicity with severely damaged glomeruli (GL), interstitial vascular congestion (V), and epithelial degeneration (ED), dilatation of renal tubules. e, f- sections of male pigeon administrated with EB 8 mg/kg/bw showed significant nephrotoxicity with severely damaged glomeruli (GL), interstitial vascular congestion (V), and epithelial degeneration (ED). (H & E stain: 40x, 100x).

DISCUSSION

The pesticides tend to become concentrated as they move up the food chain. They accumulate in the organism body (target and non-target organisms) which contain them. The pesticides don't kill the individual birds often but do affect their bodies so that they lay eggs with very thin shells often these thin-shelled eggs break or the birds are unable to reproduce. Pesticides also affect bird's physiological functions. The natural conservation committee and the royal society for birds protection suggested that the indirect effect of pesticides was a major cause of the decline of birds species[18]. Changes in blood constituents are routinely used to determine various states of the body for clinical purposes or physiological studies. Blood constituents can be used to determine stresses due to intoxication with pesticides and other environmental pollutants, nutritional and pathological factors.

The present data demonstrate that the body weight of male pigeons significantly decreases at a dose (4 and 8 mg/kg/bw day) of EB comparing to the control group. Repeated exposure to EB at doses 4 and 8 mg produces seizures, tremors, weight loss, or other types of apparent behavioral or physiological dysfunctions. The weight in animals serves as an index of growth rate[19]. Fourteen -week study in Sprague-Dawley rats designed to investigate the neurotoxicity of Emamectin benzoate, the rats were administrated daily with EB at doses 0.25, 1, 5 mg/kg body weight. Bodyweight gain and food consumption were significantly reduced at the high-level dose of 5 mg/kg/day[20].

Oral administration of male pigeons with Emamectin benzoate with doses 4 and 8 mg/kg/bw for 45 days significantly decreased all the investigated hematological parameters: RBCs, WBCs, Hb, MCV, HCT and Thrombocyte (Table2). The aforementioned findings are in coincidence with those reported by Eissa and Zidan[21] who proved that the high dose of abamectin (1/10 LD50) caused a significant reduction in erythrocyte counts (RBCs), leukocyte counts (WBCs) and hemoglobin concentration of treated rats. A significantly reduced amount of white blood cells could be indicative of immuno-suppression. The reduction in erythrocyte counts and consequently hemoglobin concentration may be attributed to more than one factor, i.e. the failure to supply the blood circulation with cells from hemohepatic tissues since the liver has an important role in the regeneration of erythrocyte and the possible destructive effect on erythrocyte by the toxicants[22].

The present work showed that serum urea, creatinine, total protein and albumin concentrations were increased significantly in all intoxicated groups of the male pigeons with

a repeated dose of EB (4 and 8 mg/kg/bw) for 45 days. Elevation of urea concentration in serum of treated male pigeons may be attributed to the toxic effect of EB which led to disorders of the kidney which attributed to the reduction in glomerular filtration in the kidney and also reflect dysfunction of the kidney tubules[23] and consequently retention of urea in the blood. The accomplished results closely resembled those obtained by Eissa and Zidan[24] and Hirohisa *et al.*,[25], who reported that EB has a significant increase in serum uric acid and creatinine levels in male rats administered with EB and Pyridalyl. A decreased value of total protein may reflect liver or kidney disease[26]. Hypoalbuminemia (decreased albumin) is a liver disorder thought to be a consequence of decreased hepatic synthesis of albumin[27].

Result data showed increased levels of cholesterol and triglycerides in pigeons treated with EB. These results coincide with those of Waly *et al.*,[28], who found that the serum cholesterol and triglycerides were significantly increased in rats treated orally through gavage with diazinon (10 mg/kg per day in corn oil) or propoxur (10 mg/kg per day in corn oil) for four weeks compared with control animals. Cholesterol and triglycerides levels were considered a valuable indicator of drug-induced disruption of lipid metabolism. An increase of cholesterol and triglycerides levels in pigeons suggests increased synthesis and accumulation of cholesterol and triglycerides. Accumulation of pesticides in the liver is reported to disrupt lipid metabolism and increase serum cholesterol and triglycerides[28,29]. This disruption may be due to decreased lipoprotein lipase activity in adipose tissue and increased levels of total serum cholesterol and triglycerides in the affected pigeons[28,30].

The histopathological finding showed damage in the tissue architecture of the kidney from the EB (4 and 8 mg/kg/bw) treated groups which showed wide lumina, severely damaged glomeruli, interstitial vascular congestion and epithelial degeneration, dilatation of renal tubules and highly vacuolation. Eissa and Zidan[21] concerning the kidney, Vertimec (abamectin as the active ingredient) at 1/10 and 1/100 of LD50 doses levels induced interstitial nephritis in male rat's Kidney.

CONCLUSION

The data of the present study has indicated that Emamectin benzoate has induced toxicological effects on male pigeons at 4 and 8 mg/kg/bw/day dose level respectively when exposed for 45 days. However, 2 mg/kg/bw/day for Emamectin benzoate doses have caused no adverse effects. Thus, based on parameters such as the development of signs of intoxication and mortality, organ body weight ratio, hematology, enzymatic changes and

histopathological examination of experimental male pigeons. It may be suggested that 2 mg/kg/day for Emamectin benzoate produced no desirable effects, therefore these doses may be considered as No Observed Effect Level (NOEL) for insecticides to male pigeons.

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