

## **TOXOLOGICAL AND PATHOLOGICAL STUDY OF DIAZINON ON MALE WILD PIGEON ( *Columba livia gaddi*) IN BASRAH CITY**

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### **ABSTRACT**

A five months as toxologic pathologic study of diazinon on male wild pigeons ( *Columba livia gaddi*) by oral intubation was done. The animals divided to four groups each with six pigeons: A( high dose 0.6 mg), B( intermediate dose of 0.3 mg), C( low dose 0.15 mg) and D(untreated control). Clinical observation of treated pigeons did not show significant changes, but a clear histopathological changes were founded. In livers of group A showed a periportal foci of mononuclear cells, while, another showed a septal fibrosis and foci of mononuclear cells in the same group. A foci of mononuclear cells were shown in group B. In group C there was a periportal fibrosis, congestion and foci of mononuclear cells.

A dilated cortical tubules were showed in kidney in all groups. While, in section from sciatic nerve related to groups (A-C) showed degenerated and vacuolated nerve fibers, and vacuolated nerve fibers were founded in group B and C. A several degenerate vacuolated nerve fibers were founded in the spinal cord of pigeons in A group, while, in B group a degenerate, vacuolated nerve fibers. In C group a several degenerate, vacuolated nerve fibers.

## INTRODUCTION

Diazinon, (0, 0-diethyl 0-(2-isopropyl 1-methyl 1-4-pyrimidinyl) phosphorothioate) and several other organophosphorus insecticides exert their toxic effects by inhibiting cholinesterase in many different animals ( 1).

Diazinon, an organophosphorus compound with an anticholinesterase mode of action, was released for experimental evaluation in the early 1950's. Today, diazinon is used extensively by commercial and home applicators in a variety of formulations to control flies, cockroaches, lice on sheep, insect pests of ornamental plants and food crops (especially corn, rice, onions, and sweet potatoes), forage crops such as alfalfa, and nematodes and soil insects in turf, lawns, and croplands (2; 3; 4; 5; 6).

Diazinon poisoning effects in animals and can be delayed or prevented by treatment with a variety of compounds ( 7; 8; 9). For example, AChE in diazinon-stressed birds can be reactivated by pralidoxime. Adding of tryptophan and its metabolites may prevent teratogenic defects by maintaining nicotinic adenine nucleotide (NAD) levels in diazinon-treated chicken embryos and diazinon reportedly acts to decrease the availability of tryptophan to bird embryos, subsequently interfering with NAD metabolism and causing birth defects (10). While, (9) recorded that NAD metabolism in diazinon-stressed birds may also be maintained with nicotinamide. In contrast to many other organophosphorus insecticides, organisms that survive diazinon-inhibited cholinesterase levels can undergo considerable spontaneous reactivation (dephosphorylation), indicating that its dephosphorylation occurs more readily than that of cholinesterase inhibited by other organophosphorus compounds (8).

Diazinon toxicity varies widely within and among species, and is modified by organism age, sex, body size, climatic conditions, pesticide formulation, chemistry of the environment, and other factors (11).

Diazinon has a potential for causing acute avian poisoning episodes (12). Ingestion of 5 granules of Diazinon 14G (14.3% diazinon) killed 80% of house sparrows (*Passer domesticus*), and all red-winged blackbirds to which they were administered (13). Ingestion of fewer than 5 granules of Diazinon 14G, each containing about 215 ug of diazinon, could be lethal to sparrow-sized birds (i.e., 15 to 35 g body weight), especially juveniles of seed-eaters

(13). Acute oral LD-50's indicate that 15 mg of diazinon/kg body weight is fatal to virtually all species tested, and that 2 to 5 mg/kg is lethal to the more sensitive species. Signs of diazinon poisoning in birds included muscular incoordination, wing spasms, wing-drop, hunched back, labored breathing, spasmodic contractions of the anal sphincter, diarrhea, salivation, lacrimation (tear production), eyelid drooping, prostration, and arching of the neck over the back, and most of these signs have been observed in birds poisoned by compounds other than diazinon; these compounds also act via an anticholinesterase mode of action (14).

The aim of the present study was to investigate the toxopathology of diazinon in wild pigeon in many organs at Basrah city/ southern Iraq.

## **MATERIALS AND METHODS**

### **Animals:**

Adult wild male pigeons were purchased from local market from Basrah city with average body weight ( 200-350 gm.) and reared in a clean cages ( 200 X 100 X80 cm.) in poultry unite / college of veterinary medicine/ Basrah university, all pigeons were acclimatized for 10 days before start the experiment.

### **Chemicals:**

Diazinon 60 EC was applied as a commercial emulsifiable concentrate formulation containing 60% active ingredient, 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 diazinon were determine by testing the compound on few pigeons ,also the maximum toxic dose used according to the active ingredients of the substance.

### **1- Treatments:**

To study a toxic effect of diazinon on pigeons a total Twenty four adult male wild pigeon(*Columba Livia gaddi*) weighting( 200-350 gm) were randomly allocated and housed in separate cages of the college of veterinary medicine, Basrah university. The birds equally divided into four groups: A,B.C and D(6 birds in each group). They are treated with diazinon for 150 day. Birds of the group A, B, and C were orally(a gavage needle) given daily doses of diazinon at the levels of 0.15mg, 0.3 mg and 0.6mg respectively, whereas group D was acted

as control. All of the birds were supplied with food with water *ad libitum*. The birds were killed after 150 day by cervical dislocation and the organs (brain, sciatic nerve, spinal cord, liver and kidney) were removed and fixed in 10% neutral buffered formalin for histopathological examination.

### **Histopathological Study:**

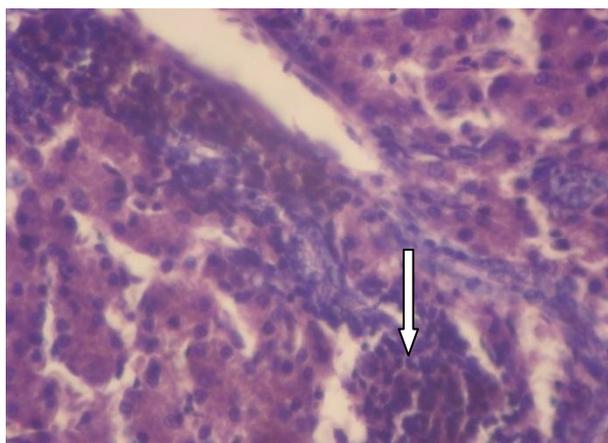
Five  $\mu\text{m}$  thick paraffin sections of 10% neutral buffered formalin as fixative, liver, kidney, brain, spinal cord and sciatic nerve from each pigeon were fixed in formalin, then samples were cut and paraffin blocks were made, sections stained with Haematoxyline-Eosin (HE), selected histopathological changes were photographed from treatment related histopathological changes in comparison to untreated controls, according to the method of (15).

## **RESULTS**

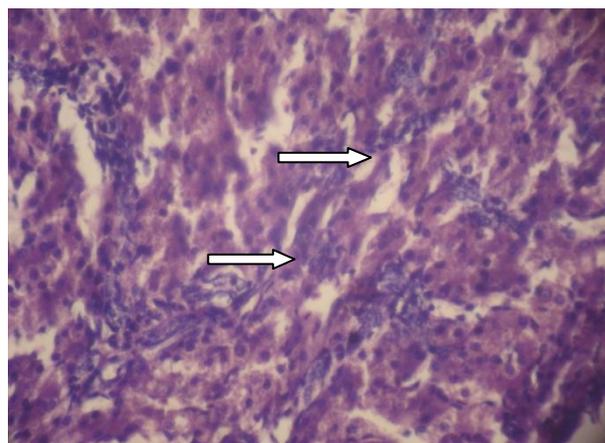
The toxicologic pathology for diazinon on pigeons were showed that:

### **1-Liver:**

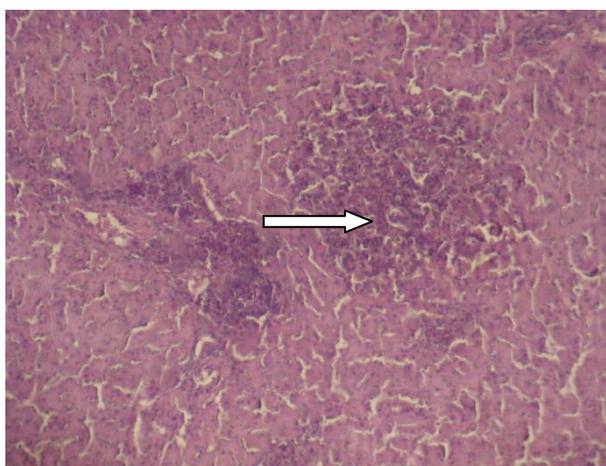
Liver in group (A) showed a periportal foci of mononuclear cells ( fig. 1), while, another showed septal fibrosis and foci of mononuclear cells in the same group ( fig. 2). A foci of mononuclear cells were shown in group( B ) (fig. 3). In group( C ) there was a periportal fibrosis, congestion and foci of mononuclear cells (fig.4), as compared with control group ( fig. 5).



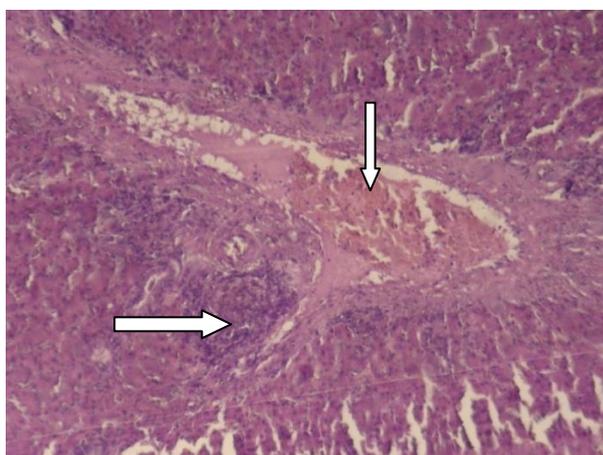
**Fig. (1):**Liver in group A with periportal foci of mononuclear cells.(H&E stain)(250X)



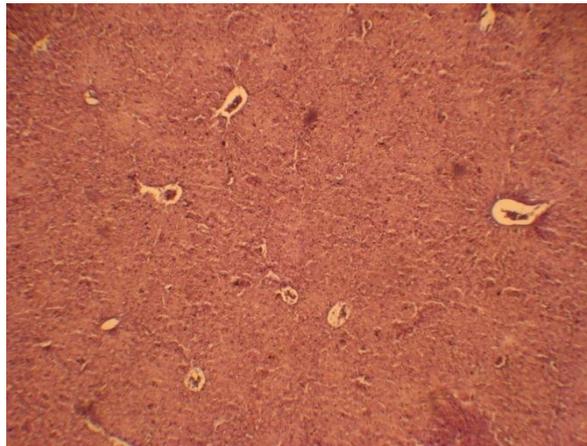
**Fig. (2):**Liver in group A with septal fibrosis .(H&E stain)(250x)



**Fig. (3):** Liver in group B a foci of mononuclear cells.(H&E stain)(125x)



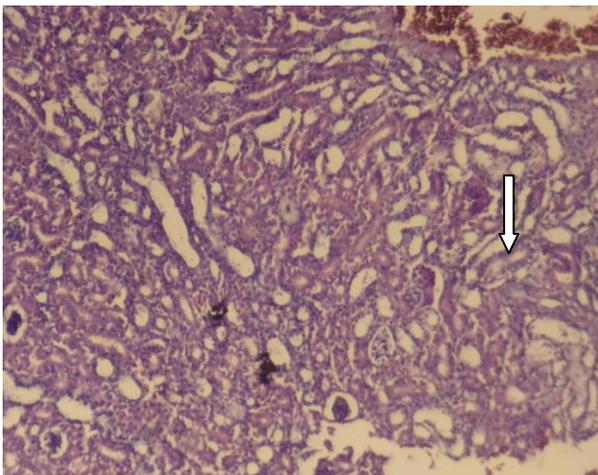
**Fig. (4):** Liver in group C a periportal fibrosis, congestion and foci of mononuclear cells.(H&E stain)(125x)



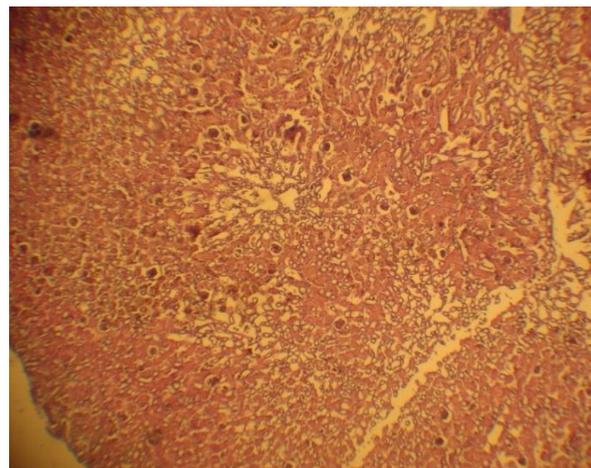
**Fig. (5): Liver in group D within normal limit.(H&E stain)(125x)**

**-Kidney:**

Kidney in light microscopy examination sections related to showed a dilated cortical tubules were found in all groups under this study ( fig. 6) as compared



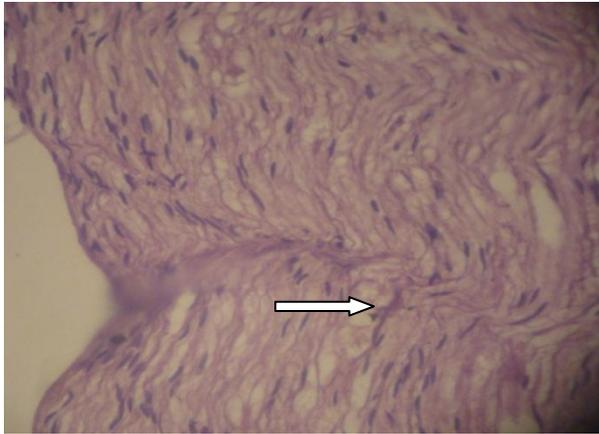
**Fig. (6): Kidney in group C: Dilated cortical tubules.(H&E stain)(250x)**



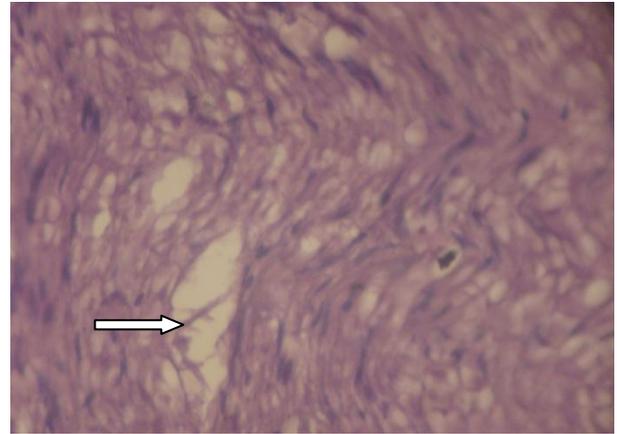
**Fig. (7): Kidney in group D within normal limit.(H&E stain)(125x)**

**3-Sicatic nerve:**

In group ( A) a few degenerated, vacuolated nerve fibers ( fig. 8).Several degenerate, vacuolated nerve fibers were found in group ( B) and( C ) (fig. 9), as compared with control ( fig. 10).



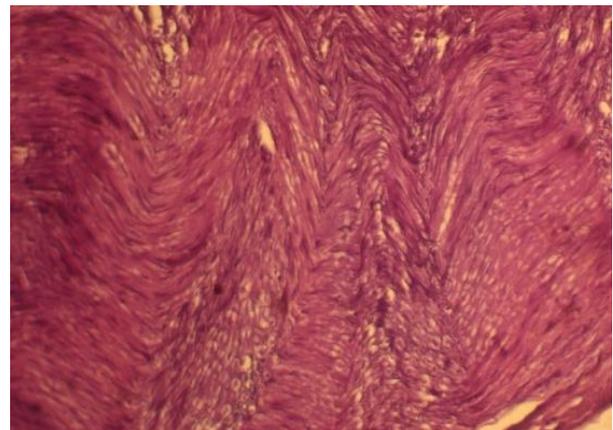
**Fig. (8):** Sciatic nerve in group A: Few degenerate, vacuolated nerve fibers.(H&E stain)(250x)



**Fig. (9):** Sciatic nerve in group B: A degenerate, vacuolated nerve fibers.(H&E stain)(250x)



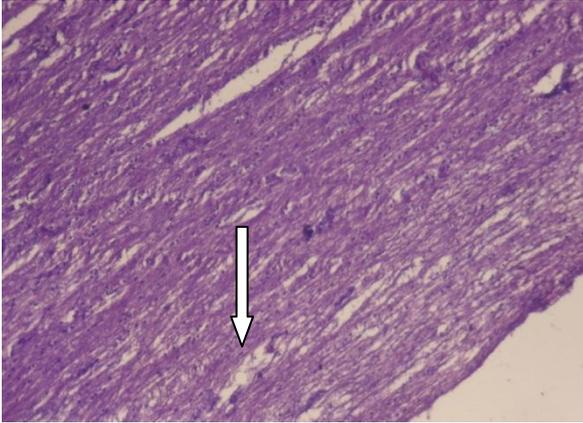
**Fig. (10):** Sciatic nerve in group C: Several degenerate, vacuolated nerve fibers.(H&E stain)(125x)



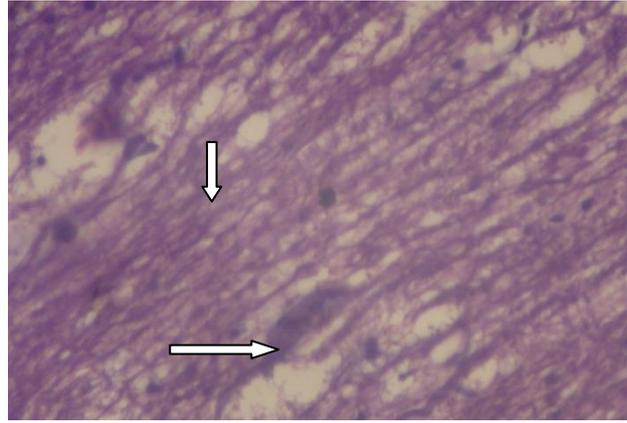
**Fig. (11):** Sciatic nerve in group D within normal limit.(H&E stain)(125x)

#### 4-Spinal cord:

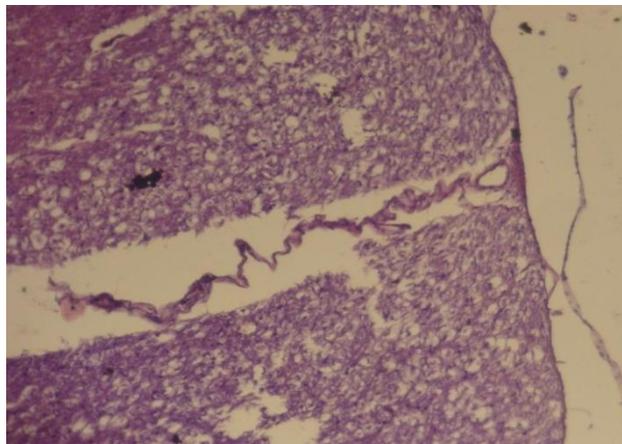
Several degenerate vacuolated nerve fibers were found in the spinal cord of pigeons in( A) group ( fig. 12), while, in( B) group a degenerate, vacuolated nerve fibers (fig. 13). In C group several degenerate, vacuolated nerve fibers ( fig. 14).



**Fig. (12): Spinal cord in group A: Several degenerate, vacuolated nerve fibers.(H&E stain)(125x)**



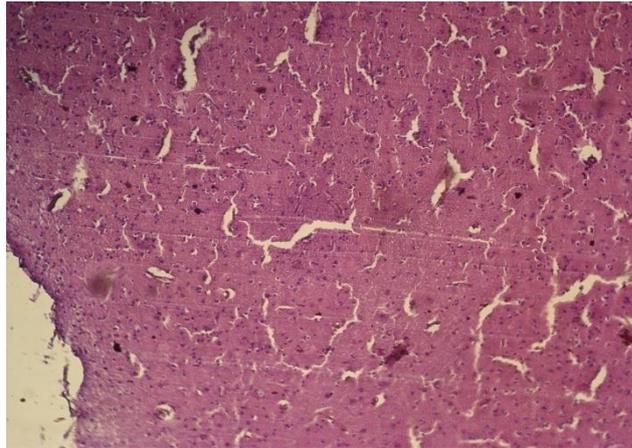
**Fig. (13): Spinal cord in group B: Several degenerate, vacuolated nerve fibers.(H&E stain)(250x)**



**Fig. (14): Spinal cord in group C: Several degenerate, vacuolated nerve fibers.(H&E stain)(125x)**

### **5-Brain:**

The brain of all pigeons for all groups under this study without any pathological changes, as shown in ( fig. 15).



**Fig. (15): Brain without any changes in group C within normal limit.(H&E stain)(125x)**

## **DISCUSSION**

Diazinon is a very highly toxic organophosphate compound. Organophosphates are long known and widely applied active ingredients of different insecticides used in the plant protection practice. In animal production these compounds are used to control a variety of ectoparasites such as mites and lice. In the living organism, organophosphates caused inhibit the enzyme acetylcholinesterase, causing an accumulation of acetylcholine, a neurotransmitter involved in impulse transmission, and leading to an over-stimulation of the parasympathetic nerves (16). Poisoned animals show salivation, lachrymation, diarrhoea and convulsions followed by depression, prostration, ataxia and cyanosis, and then death usually ensues within a short time. Avian species are more susceptible to the toxic effects of diazinon than are mammals. Poisoned chickens often exhibit only respiratory distress, lachrymation and salivation before death, and therefore the suspicion of an acute respiratory infection may also arise (17). Diazinon is rapidly metabolized in mammals and is excreted principally through the urine. It is metabolized in vivo by four enzyme systems, which include mixed function oxidases, hydrolases or phosphatases, glutathione-dependent transferases, and non-specific esterases. Most in vivo animal studies have demonstrated the production of diazoxon, hydroxydiazinon, isohydroxydiazinon, and a propylenediazinon metabolite. Diazinon does not bioaccumulate in tissues or organs. The mode of action of diazinon, as with other organophosphate insecticides, is inhibition of the enzyme cholinesterase ( 18).

There was a pathological changes in different organs of pigeon under this study; in liver found a fibrosis in many parts and periportal foci of mononuclear cells. This is because that which could be due to the toxic effects of diazinon on liver cells due to the metabolic mechanisms of the liver cells, and the vacuolation of cytoplasm of hepatocytes may be from extensive lipid infiltration. This result agree with many studies on different animals by ( 19; 20).

(21; 22) noticed 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 and this is because a toxic agents as carbon tetrachloride, phosphorus and chlorinated hydrocarbon insecticides.

Another study reported that diazinon cause an demonstrated the signs of circulatory disturbances in the inner organs of geese (23). Diazinon is highly toxic to birds and the acute oral LD50 (mg/kg) for technical diazinon is: 6.81 for turkey; 40.7 for chicken; 14.7 for goose; 2.75 for gosling. The sub acute dietary LC50 (ppm) for technical diazinon is: 191 for mallard ducks; 245 for bobwhite quail; 244 for ring-necked pheasant; 47 for Japanese quail (18).

In kidney the main pathological signs was a dilated cortical tubules, this may be that diazinon cause a toxicity in renal system by their metabolism of this compound and the immune system make a good role for defending against foreign particles, as it was found in the present study.

In sciatic nerve and spinal cord the main changes was a degenerate in fibers this may be due that diazinon cause a neurotoxic effects on these organs.

Diazinon exposure of pregnant laboratory animals in testes has demonstrated that this insecticide can cause a variety of reproductive problems, including damage to the developing nervous system, delays in sexual development, stillbirths, death of newborn offspring, and birth defects. But the effects on the developing nervous system are most significant( 24).

(23) reported that a histopathological examination of Geese with diazinon poisoning was demonstrated changes indicative of acute circulatory disturbance, passive congestive hyperaemia and, occasionally, mild interstitial edema was observed in the brain, liver, kidney, heart and pancreas.

## **ACKNOWLEDGMENT**

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## دراسة أمراضية سمية للديازينون في ذكور الحمام البري في مدينة البصرة

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### الخلاصة

اجريت دراسة امراضية - سمية على ذكور الحمام البري في مدينة البصرة لمدة خمسة اشهر وذلك بواسطة التجريع الفموي لمادة الديازينون، حيث قسمت التجربة الى اربع مجاميع كل مجموعة تتكون من ستة طيور: المجموعة الاولى أ (الجرعة العالية 0.6 ملغ)، المجموعة الثانية ب (الجرعة المتوسطة 0.3 ملغ) اما المجموعة الثالثة س (الجرعة الواطئة 0.15 ملغ) و المجموعة الاخيرة د (السيطرة).

لم تظهر العلامات السريرية للطيور المعاملة أي تغيرات واضحة ولكن ظهرت تغيرات نسيجية مرضية واضحة، حيث لوحظت تغيرات على اكباد الحمام المجرع بالديازينون منها: البؤر امام البوابية و ارتشاح خلايا التهابية وحيدة النواة، بينما اظهرت طيوراً اخرى تليفا بين الفصوص وبؤر من خلايا التهابية.

لوحظ على الكلى تغيرات متمثلة بتوسع النبيبات الكلوية في منطقة القشرة في جميع المجاميع المعاملة. اما العصب الوركي فلو حظ عليه تنكس فجوي يتراوح ما بين القليل والمتعدد في الالياف العصبية بين المجاميع المعاملة وكذلك هو الحال في الحبل الشوكي. اما الدماغ فلم يلاحظ عليه أي تغيرات مرضية في جميع المجاميع المعاملة في الدراسة الحالية.

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