STUDY THE TOXOPATHOLOGICAL EFFECTS OF NEOCIDOL ® (25% DIAZINONE) IN BROILER CHICKENS

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ABSTRACT
This study was designed to identify the toxopathological effect of Neocidol ® (25% Diazinone) on chicken organs. For this reason , the sublethal dose of this insecticide was 0.37mg / chicken ( 1Kg.B.Wt) , whereas , 5mg, 7.5mg & 15mg / chicken were toxic caused the death of the chicken within 1-2 hrs postoral administration of the insecticide whereas, all the chicken administrated the sublethal dose (0.37mg/ chicken ) were survived for 10-14 days, and showed the different clinical signs included dyspnea, ataxia, paralysis, lacrimation, salivation, diarrhea, tremors, icodination, dullness and depression. Different irreversible degenerative changes were recorded in the different organs of the chicken, among the degenerative changes included the cloudy swelling, hydropic degeneration, necrosis and congestion and hemorrhage in liver and kidneys. Also hemorrhage and congestion were seen in between myocardial muscle fibers. Lymphoid depletion and hemorrhage and congestion together with edema were seen in bursa fabricious and spleen. Also depletion of lymphoid tissue of intestinal peyer’s patches and peribronchial lymphoid tissue of the lungs together with congestion, edema and hemorrhage. Testicular degeneration and edema and congestion were seen in testicular tissue. Also degeneration and demyelination of the peripheral (sciatic nerve) together with perineuronal edema, congestion and perivascular leukocytes cuffling were seen in brain tissue.

KEYWORDS: insecticide, dyspnea , ataxia , paralysis , lacrimation , salivation , diarrhea , tremors, perivascular leukocytes.

INTRODUCTION
Neocidol® (25% Diazinone) is a commercial thio phosphate Insecticides ordinary used for chicken premises proved highly toxic when used in a pen housing 1). These products inhibit acetylcholinesterase, causing acetylcholine to accumulate, when results in the overstimulation of parasym pathetic nerves and muscles . Among the organophosphorus compounds, the Diazinone is used to control fire ants and darkling beetles, but in birds, diazinone can cause incardination, paralysis, respiratory signs and deaths 2). It is also used to control pests in soil and grass, causing death of birds 3). Some organophosphates and carbamates have delayed neurotoxic effects. Chickens and other birds are more susceptible than mammals to this type of toxicity 4). No specific microscopic changes have been identified in chickens for this reason this study aimed to identify the sublethal toxic dose of Neocidol® (25% Diazinone) in broilers chickens and identify both clinical signs and pathological findings associated with experimental oral administration of sublethal toxic dose of Neocidol® (25% Diazinone).

MATERIALS & METHODS
Preparation of sublethal toxic dose; Neocidol® (25% Diazinone): Different concentrations have been prepared by mixing the suitable amount of Neocidol® (25% Diazinone) a commercial thio phosphates insecticides with corn oil for administration orally. The following dose levels to four groups of chicken aged 6 weeks at average weight of 1Kg

1- 1st group 3.75 mg/ chicken
2- 2nd group 5 mg/ chicken
3- 3rd group 7.5 mg/ chicken
4- 4th group 15 mg/ chicken

During this pilot study 5) the sublethal dose was 3.75 mg/ chicken. All the birds were kept for waiting for 10-14 days and all the clinical signs appeared on birds were recorded and for histopathology, small pieces of tissues from different organs were taken in 10% Neutral buffered formaline for fixation and histological sections were performed and examined under light microscope 6).

RESULTS
The sublethal dose
It was 3.75mg/kg (chicken, 1Kg b.wt.) whereas, the doses 5 mg/ chicken, 7.5 mg / chicken and 15 mg / chicken) were lethal for the chickens, cause death of the chickens within 1-2 hrs.

Clinical signs
All the chickens taken toxic doses (5 mg, 7.5 mg and 15 mg/ chicken) were died quickly, showing few signs of dyspnea, paralysis and death within 1-2 hrs. But all the chicken taken sublethal dose showed dyspnea, paralysis, lacrimation, salivation, diarrhea, tremors, depression, dullness, lethargy, cyanosis, ataxia, in coordination and convulsion. All these clinical signs persisted on chicken for 10-14 days post oral administration the sublethal dose of Neocidol® ( 25% Diazinone), some of these clinical signs were gradually decreased at last period ( 14th day ).
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post oral administration of sublethal dose of the Neocidol® (25% Diazinone).

**Histopathological changes**

- **Liver:** It showed variable irreversible degenerative changes and regeneration response mediating defense mechanism. Among the degenerative changes were cloudy swelling, hydropic degeneration of some hepatocytes (Fig-1), congestion of the central veins and sinusoidal dilation. Apoptosis of some hepatocytes and necrosis and hypertrophy of other hepatocytes, at the same time hepatocytes loss their polygonal structure and mild aggregation of the mononuclear cells (lymphocytes, macrophages and plasma cells) in the portal region.

![FIGURE 1 Liver: hydropic degeneration and sinusoidal dilation (H&E) x400](image1)

KIDNEYS: It showed different degenerative changes among these changes were cloudy swelling and hydropic degeneration (Fig-2) and necrosis of epithelia lining the proximal convoluted tubules, congestion of glomeruli and all the renal blood vessels. Also mild mononuclear cells infiltration at the interstitial renal tissue together with Baumans space dilation.

![FIGURE 2 Kidney: hydropic degeneration, necrosis of renal tubular epithelia and dilation of Bauman's Space (H&E) x400](image2)

- **Heart:** It showed mild degenerative changes of the myocardial muscles fibers together with sever congestion and hemorrhage between muscle fibers in the myocardium and epicedium (Fig-3).

![FIGURE 3 Heart: hemorrhage between muscle fibers, edema and congestion (H&E) x400](image3)
**Bursa fabricious:** It showed depletion of their cortical lymphoid tissue (Fig-4) together with edema and congestion of all the bural tissue.

![Bursa fabricious](image)

**FIGURE 4** Bursa fabricious: Depletion of cortical lymphoid tissue and edema (H&E) x 400

**Spleen:** It showed lymphoid depletion of the white pulp region together with hemorrhage, congestion and hemosidrosis in the white and red pulps (Fig-5).

![Spleen](image)

**FIGURE 5** Spleen: Depletion of white pulp and hemorrhage (H&E) x400

**Lungs:** It showed extensive pulmonary edema and congestion and depletion of the peribronchial lymphoid tissue (Fig-6).

![Lungs](image)

**FIGURE 6** Lungs: pulmonary edema and congestion and hemorrhage (H&E) x100

**Intestine:** It showed sloughing of their mucosal epithelia together with congestion and hemorrhage in mucosal and submucosa of the intestine (Fig-7). Also depletion of the peyer's patches along the intestinal mucosa together with mucinous degeneration.
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**FIGURE 7** Small intestine: hemorrhage, congestion at the mucosa, submucosa and muscular layer (H&E) x100

**Testes:** It showed loss of spermatogenesis, hydropic degeneration of all the germinal layers of seminiferous tubules (Fig- 8) together with edema and congestion of the interstitial testicular tissue.

**FIGURE 8** Testis: Testicular degeneration, edema and loss of spermatogenesis (H&E) x100

**Brain and peripheral (sciatic nerve):** It showed extensive congestion of the blood vessels together with mild perivascular leukocytes cuffing and perineuronal edema (Fig- 9).

**FIGURE 9** Brain: perivascular leukocyte cuffing, perineuronal edema and congestion (H&E) x400.

Sciatic nerve showed extensive degeneration and demyelination of the nerve fibers and edema (Fig-10).

**FIGURE 10** Sciatic nerve: degeneration, demyelination of nerve fibers and edema (H&E) x100
DISCUSSION
The sublethal dose of the Neocidol® (25% Diazinone) was 0.37 mg/chicken, whereas, 5mg, 7.5 mg & 15 mg/ chicken were toxic to all chickens, the amount of sublethal dose were varied between species of animals and other species and even, in the same species[6] there was variation in the sublethal and toxic dose of the insecticides, these variations depend on the age of animals and chemical components of the insecticides[7]. Neocidol® (25% Diazinone) used in this study with the sublethal dose (0.37 mg / chicken) and induced different clinical signs mainly dyspnea, paralysis, lacrimation, tremors, diarrhea, depression, dullness, lethargy, cyanosis, ataxia, incoordination, convulsion and salivation, these clinical signs depend on the distribution of the insecticide into all the different organs of the chickens and even into the brain and peripheral nerve ( sciatic nerve ) resulted to nervous signs [8]. The histopathological findings were present in the all organs of the chickens and their distribution were similar to the hypoxic and anoxic injuries by any of organophosphorous insecticides[9]. All the pathological lesions were depended on the type of organophosphorous compound and the duration of the intoxication and amount of time lapsed between exposure and clinical signs appearance[9].

Liver showed different degenerative changes such as cloudy swelling, hydropic degeneration and even necrosis of some hepatocytes, these degenerative changes resulted from the direct toxic injury of the Diazinone which induces hypoxia / anoxia in liver cells and causes the lesions [10], together with the liver is considered the first forget organ for detoxication [10]. Similar findings were found in kidneys these organs considered also the target organs for metabolism of the toxin[11]. Diazinone in this study and therefore cloudy swelling, hydropic degeneration and necrosis of epithelial lining the proximal convoluted tubules, also the kidneys received large amount of blood flow as a major role of excretion of toxic materials [11], so higher effects of toxic compounds were observed. Other lesions such as hemorrhage, edema and degeneration were observed in the myocardium and epicardium, these vascular lesions were belonged to the direct effects of toxic compounds[2,12]. Diazinone in this study induced these vascular changes through the effect of hypoxia and anoxia which caused the damage to vascular endothelia [12]. Depletion of the lymphoid tissue of the white pulp of spleen, bursa fabricious together with edema and hemorrhage and congestion occurred as a result of hypoxia and anoxia Induced by Diazinone toxicity in this study, it is known that all the toxic insecticides and poisons had a direct effect on the lymphoid tissue and resulted into depressed immune response [6]. Also, depletion of peyer's patches in intestine and peribronchial lymphoid tissue of the lung together with congestion, hemorrhage and edema in these two organs occurred as a result of the hypoxia, anoxia occurred by the effect of Diazinone on these organs [12,13]. Also direct effect of Diazinone on intestinal epithelia lead to mucinous degeneration following the oral administration, then this insecticide absorbed into the blood and into the all organs [2]. Testes were also affected by the toxic compounds of the Diazinone and showed testicular degeneration of epithelia lining seminiferous tubules, interstitial testicular edema and loss of spermatogenesis which induced by hypoxia and anoxia as a result of the toxic effects of Diazinone compounds [5, 6], similarly to the effects of other toxins and poisons [13]. Brain and peripheral ( sciatic nerve ) also affected by the toxic components of the Diazinone through the hypoxia and anoxia induced by this insecticide lead to demyelination & degeneration of the nerve fibers [14] and perineuronal edema, congestion and perivascular leukocytic cuffing around blood vessels of brain tissue, for this reason paralysis, ataxia, incoordination and dullness occurred as a delayed neurotoxicity by this insecticides similar to other insecticides effects in chickens and other birds [15].

REFERENCES
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