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Neurolgical Effect of Three Organophosphorus Compounds in Adult Hens, 1- Acute toxicity

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Abstract. This study was conducted to investigate the acute toxicity of 3organophosphorus insecticides [Diazinon, Dichlorvos, and Tri-ortho- cresyl phosphate (TOCP)] on the nervous system of adult laying hens.The clinical signs, gross lesions and histopathological changes of the nervous system have been discussed. Twenty four layer hens of Lohmen breed, 54 weeks old and 1.5kg average body weight were used for this purpose. The birds were equally divided into 4 groups. Three groups were administered 0.8, 320 and 750 mg/kg body weight of Diazinon, Dichlorvos, and TOCP respectively and fourth one was acted as control. Administration has been performed via oral gavage. Administration of these compounds resulted in muscarinic signs of toxicity which included, ruffled feathers, salivation, gasping and diarrhea, and nicotinic signs which represented by tremor, droppy wings, ataxia, resting on the sternum, leg paralysis, and recumbency. These signs were differed in their duration of appearance, severity and disappearance according to the type of OP compound. Diazinon treated group exhibited more severe signs than Dichlorvos, whereas TOCP did not produce these signs. Post-mortem examination did not reveal any gross lesion in the brain, spinal cord and sciatic nerve.Histopathological changes showed vaccualation of nerve axons in the spinal cord and the distal part of sciatic nerve due to the axonal degeneration. These changes were more severe in Diazinon followed by Dichlorvos and TOCP respectively. Acute neuronal toxicity of these compounds exhibited severe histopathological ulterations in the sciatic nerve than those noted in the spinal cord. The brain did not show any obvious changes at these levels of doses.

Key words: Diazinon, Dichlorvos, and Tri-ortho- cresyl phosphate (TOCP)

This article is a part of a thesis of the first author.

Introduction

Organophosphate (OP) insecticides are widely used in public health, veterinary practice and agriculture. They are common in the Middle East, including Iraq. Their main mechanism of toxic action in mammals and birds is to inhibit the target enzyme chlinesterance (ChE), which leads to accumulation of acetylcholine at the nerve terminals and neuromuscular junctions, and to cholinergic overstimulation manifested as muscarinic, nicotinic, and central nervous system effects (10).

Many organophosphorus compound (OPC), neurotoxic demyelinating agents, cause a specific secondary type of demyelination in the nervous tissue. A lesion of neuronal soma, axis cylinder and myelin sheath in the central and peripheral nervous system has been demonstrated. Toxicity of TOCP, a causative agent of an accidental flaccid motor paralysis with ataxia in humans, was reported in the USA (1930) and in Morocco (1958-1959) (16).

Subsequently, the toxic influence of TOCP of the nervous system in experimental animals has been described, most frequently in rats and in the most susceptible animals-hens (15; 6).

In birds the ascendant and descendent long spinal tracts were

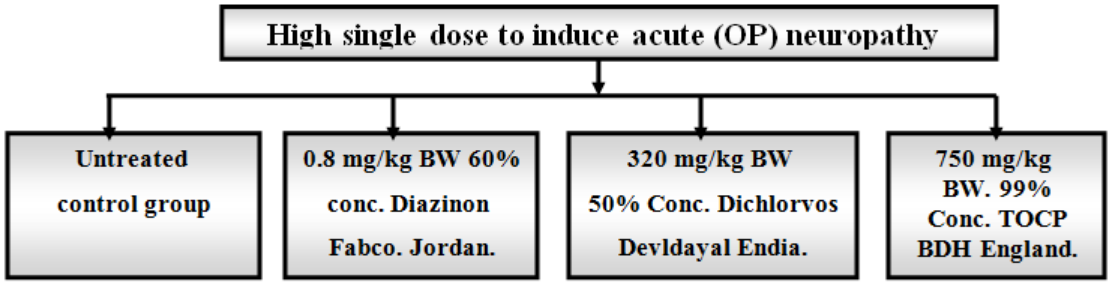
affected, but the most striking damage was observed in the peripheral nervous system in both myelinated and ummyelinated fibers (17).

The aim of this study was to induce the acute Organophosphorus (OP) neuropathy of three commonly used preparations of insecticides namely: Diazinon, Dichlorvos and Tri-orthocresyl phosphate (TOCP) in adult laying hens through oral administration of high single dose.

Materiel and Methods

Twenty four Lohmen breed at 54 weeks old and about 1.5kg average body weight layer hens were used in this study at the Department of Pathology and Poultry Diseases, College of Veterinary Medicine, Basrah University. The birds were equally divided into 4 groups. Three groups were administered 0.8, 320 and 750 mg/kg body weight of Daizinon, Dichlorvos, and TOCP respectively, and the fourth one was acted as control as showed in table (1). Administration has been performed via oral gavage. Daily observation of clinical signs has been conducted, and 21 day PA these birds have been decapitated and subjected for post-mortem examination and samples from brain, spinal cord, and sciatic nerve were taken for histopathlogical sectioning (8). The LD_{50} of each compound was used according to Hill, *et al.* (7); Wolfe and Kendall(18); Seth, *et al.* (13); Wu and Leng (19); Shaheen, *et al.* (14); Zhao, *et al.* (20).

Table (1): Experimental Design



- **Each group was consisted of (6) adult hens.**
- **A 21 day PA, brain spinal cord and sciatic nerve samples were selected for histopathological examination.**

The Maximum Tolerated Doses (MTD) of these 3 compounds were estimated through several experiments conducted on other birds of the same breed, age, and body weight until obtaining these levels of tolerated doses.

Results and Discussion

Acute toxic neuropathy induce by high single oral doses of both Diazinon and Dichloroves in the adult hens of the present study resulted in muscarinic signs of toxicity (Fig.1) which included ruffled feathers especially at the neck region, severe salivation, gasping, lacrimation, and profused watery diarrhea, followed by nicotinic signs

which characterized by tremors, in coordination, droppy wings, inability to stand, resting on the lock joints, sitting on the sternum, leg paralysis and recumbency, followed by gradual recovery. The clinical signs were detected 15-20 minutes PA of these two compounds and peaked and receded in a period of 35-40 minutes. Disappearance of these signs and complete birds recovery were seen within a range of 9-15 hours. TOCP did not exhibit noticeable sings of acute neurotoxicity. These sings were in agreement with those of Abou- Donia (1) who stated that OP compounds has a suppressive effect on AChE which results in physiological disturbance of the nervous system in a mixed breed of hens at 6-8 weeks old treated with 0.79,240 and 690 mg\kg body weight of these compounds respectively.

Patton *et al.* (11); Mohammed *et al.* (10) stated that these neurotoxic manifestations occur due to the irreversible inhibitory effect of OP on the AChE activity and accumulation of ACh at the nerve endings.

Hill *et al.* (7); AL-Ias (2) also mentioned that chickens and pigeons treated with OP compound exhibited both muscarinic and nicotinic signs of toxicity due to inhibition of AChE activity.

The Present study revealed clear differences in duration of appearance, severity and disappearance of these signs according to the type of compound. This result was in concordance with that of Mohammed *et al.* (10) who concluded that Diazinon has more toxic potential due to its increase ability for ChE inhibition and high metabolic activity with subsequent development of nicotinic, muscarinic, and central nervous effects than Dichlorvos.

Complete recovery of hens in the present study was greatly differed from that of Mohammed *et al.* (10) who stated that, two, and three out of six broiler chicks has been died after treatment with Dichlorvos and Diazinon respectively. This may be related to age, breed of chickens and dose of compound.

In the course of an acute OP – induced toxic neuropathy in hens following administration of the three insecticides, the present study did not exhibit any obvious gross neuronal lesions. This result was in agreement with those of Lotti (9); Hill *et al.* (7); Varsik *et al.* (17) who postulated that OP compounds has no chance to induce gross neuronal lesions because of short period of effect of these substances on the nervous system and their specificity to produce histopathological changes only.

The histological assessment of nervous tissue of the present study did not reveal any obvious structural changes in the brain (Fig.5). This result has in agreement with those of Cavanagh (3) and Schmidt *et al.* (15) who supposed that short time of exposure and decrease quantity of brain lipid are the major etiology of an absence of visible changes.

The Major histologic alterations were found in the spinal cord (Fig.6, 7, and 8) and peripheral nerves especially in the distal part of sciatic nerve (Fig.9, 10, and 11) which were consisted of axonal fragmentation and demyelination, vacuolation, degeneration, and atrophy of nerve tissue in compared to control group (Fig.2, 3 and 4). These results were in concordance with those of Abu-Donia (1); Varsik *et al.* (17); Zhao, *et al.* (20); Coleman and Freeman (5) who concluded that OP pesticides and their neurotoxic demyelinating influence was demonstrated in birds and the ascendant and descendent long spinal tracts were affected, but the most striking damage was observed in the peripheral nervous

system in both myelinated and unmyelinated fibers.

Cavanagh (4) described a parallel between the alterations of peripheral nerves and typical wallerian degeneration and interprets this phenomenon as a disease of the whole neuron, a process of retrogression.

Varsik *et al* (17) demonstrated that the distal type of OPC toxic neuropathy with proliferation of Schwan's cells and connective tissue, and infiltration of macrophages has also been reported, whereas the present study was lack of these changes.

The present study revealed that oral administration of Diazinon Dichlorros and TOPC induced both muscarinic and nicotinic signs of toxicity in the layer hens, but no gross lesions have been noticed in the nervous system. Histapathology vacculation of the nerve axons in the spinal cord and sciatic herve was evident. These changes were more severe in Diazinon followed by Oichlorvos and TOCP respectively.

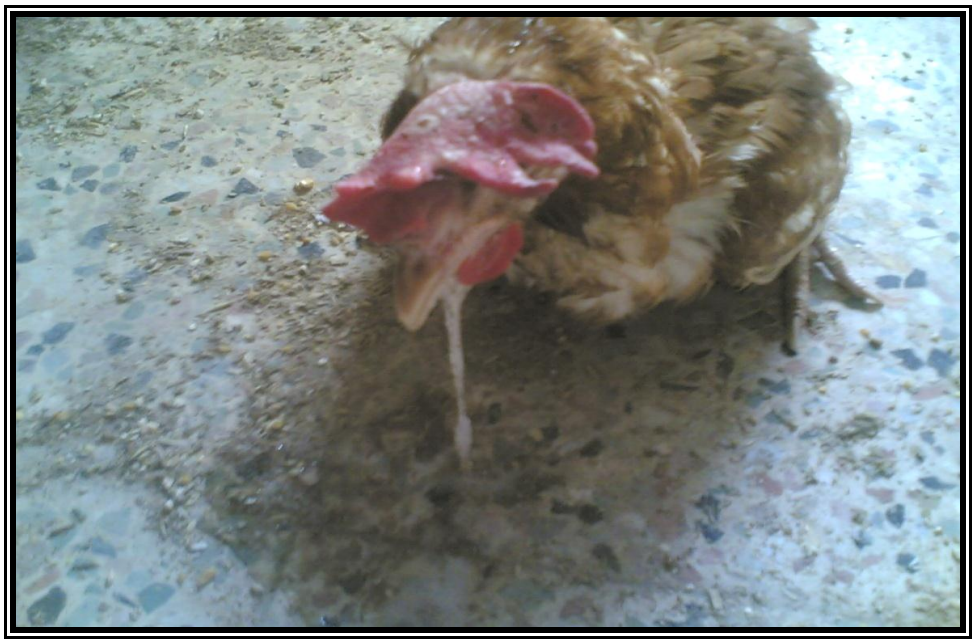


Fig. (1): Adult hen exhibited signs of toxicity.

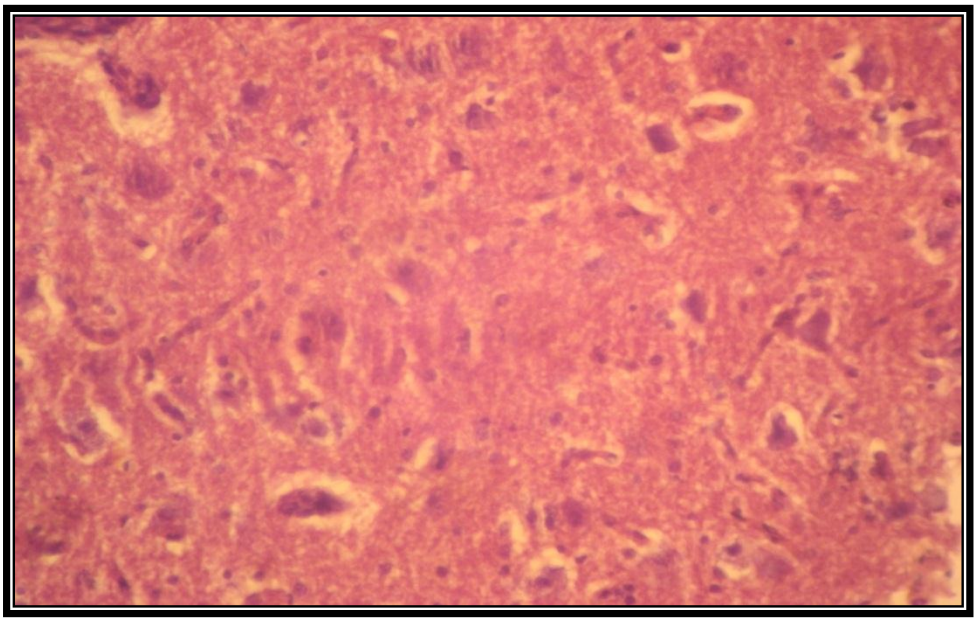


Fig. (2): Longitudinal section in the brain of hen. Control group H&E 400X.

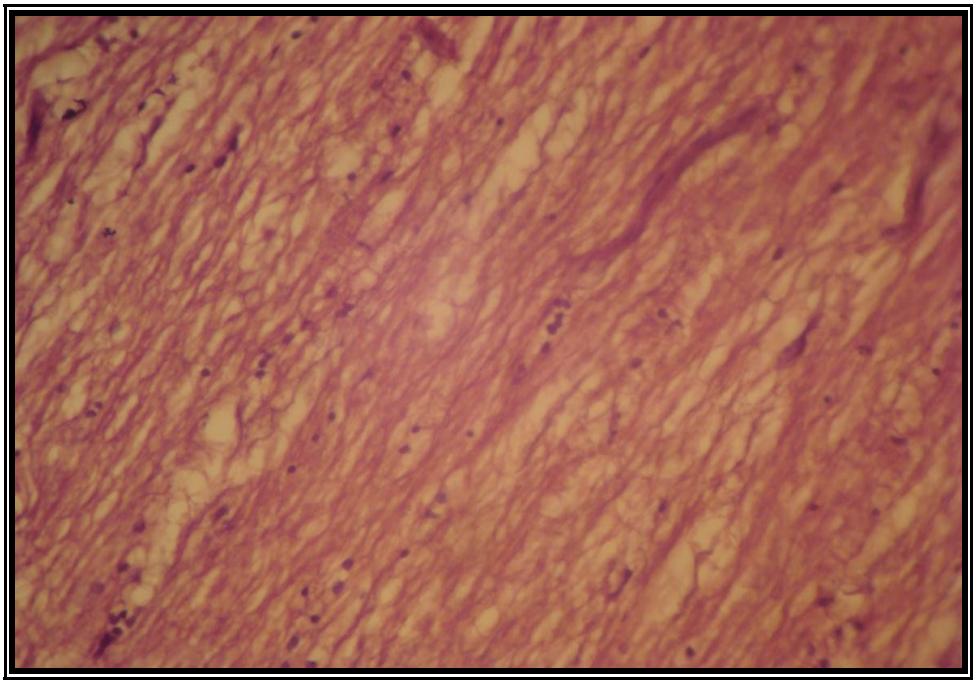


Fig. (3): Cross section, spinal cord, adult hen of control group H&E 400X.

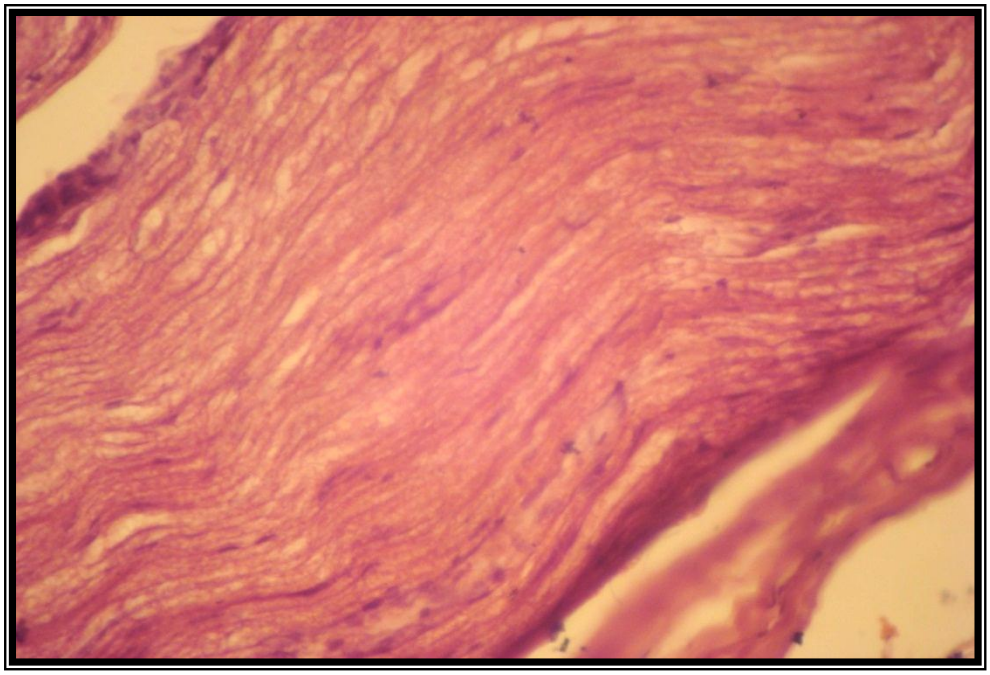


Fig. (4): Longitudinal section, sciatic nerve, control group H&E 400X.

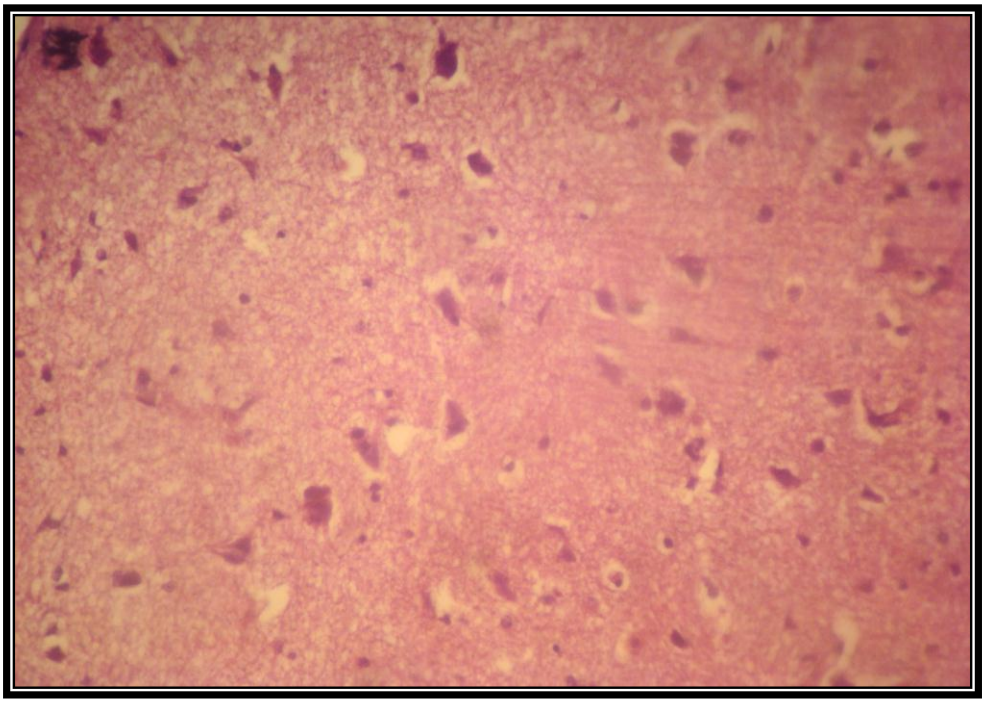


Fig. (5): Longitudinal section in the brain of hen treated with Diclorvos showed no changes H&E 400X.

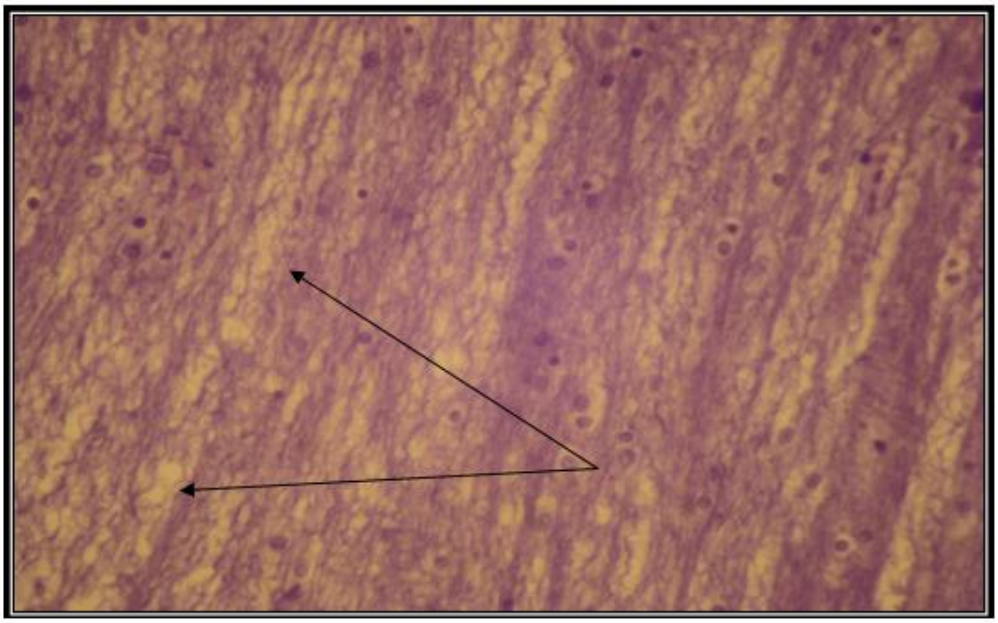


Fig. (6): Longitudinal section in spinal cord of hen treated with 0.8 mg/kg Diaznon showed slight vacuolation H&E 400X.

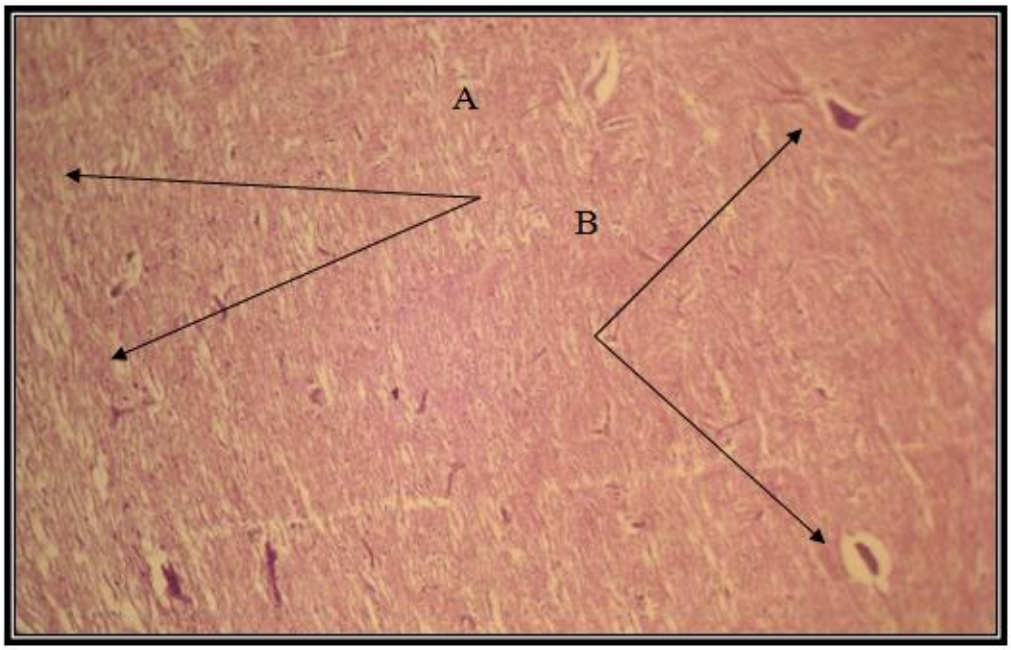


Fig. (7): Longitudinal section in the spinal cord of hen treated with 320 mg/kg Diaclorvos showed A. Slight vaculation B. Atrophy of nerve cell.

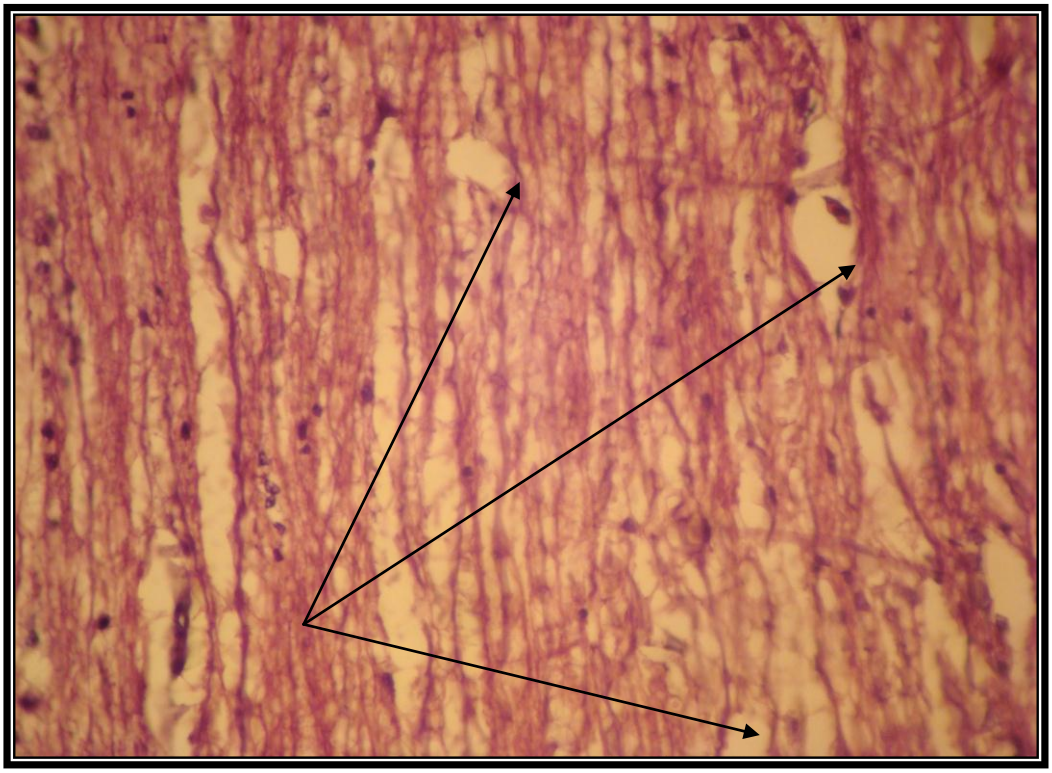


Fig. (8): Longitudinal section in a spinal cord of a hen treated with 750 mg/kg TOCP showed fragmentation of nerve fibers H&E 400X.

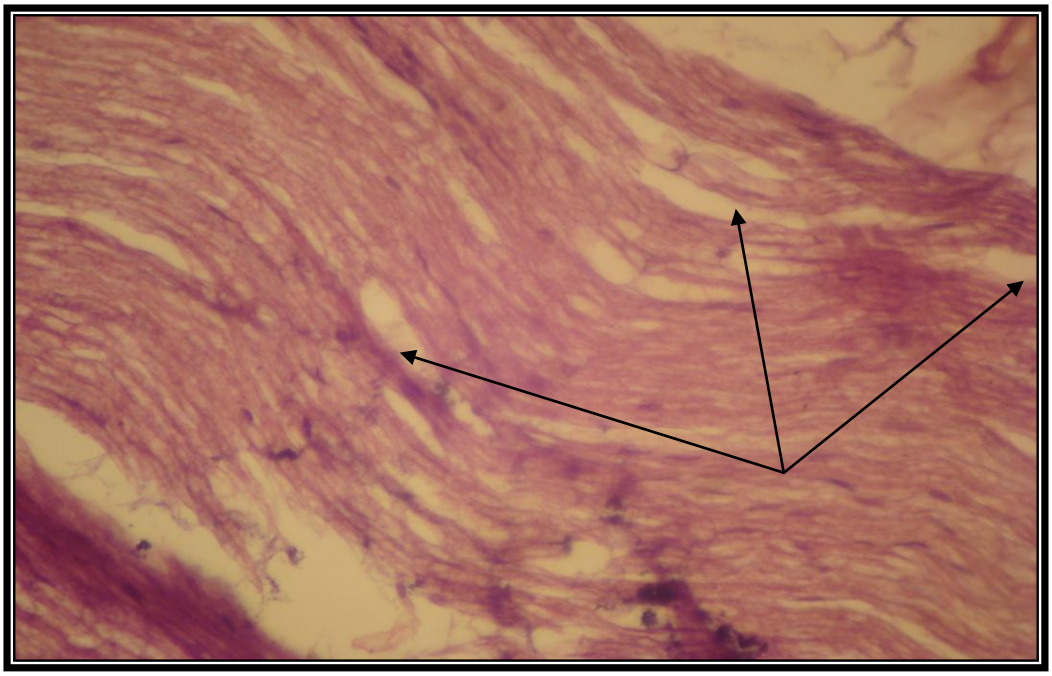


Fig.9:- Longitudinal section in the sciatic nerve of hen treated with 0.9 mg/kg Diazinon showed fragmentation of nerve fibers H&E 400X.

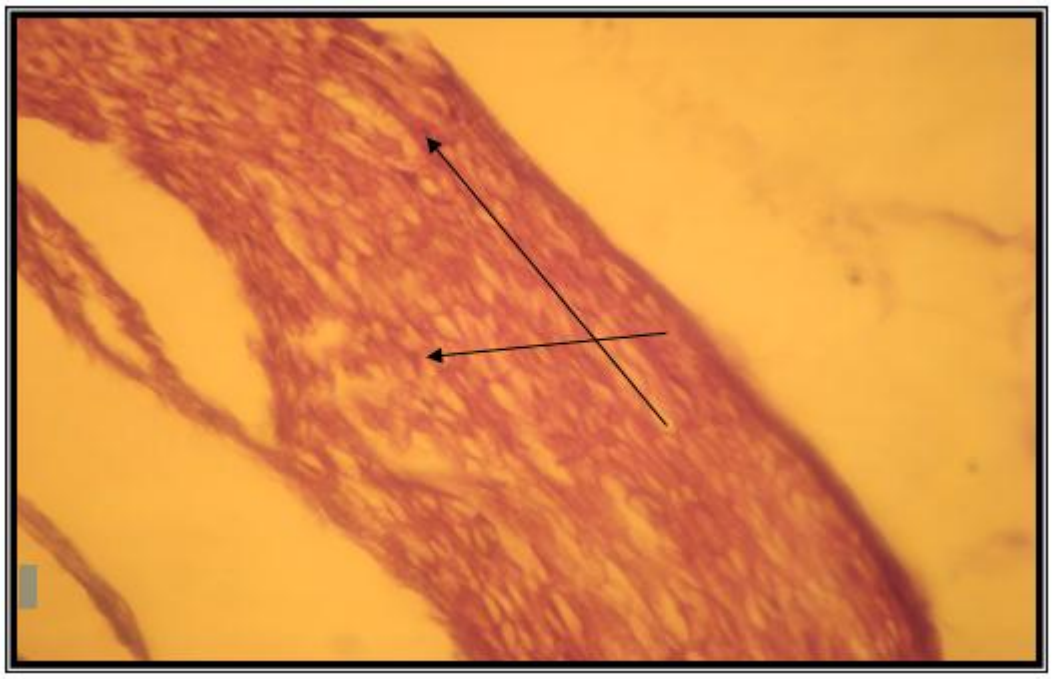


Fig. (10): Longitudinal section in the sciatic nerve in a hen treated with 320 mg/kg Diachlorvos showed slight fragmentation in the nerve fibers H&E 100X.

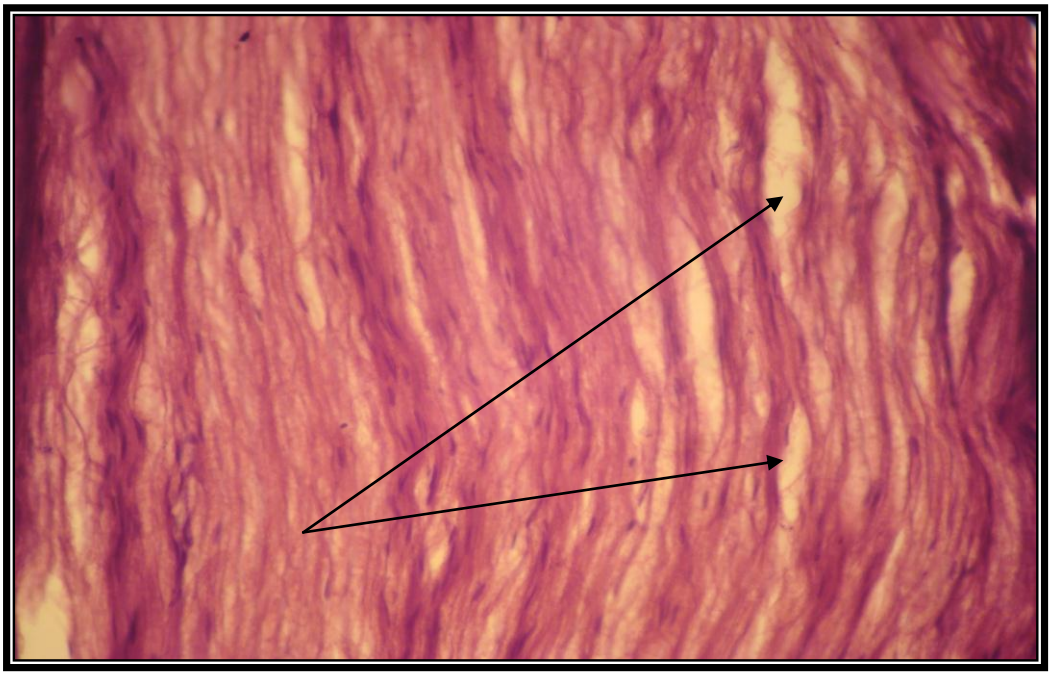


Fig.11:- Longitudinal section in the sciatic nerve of a hen treated with 750 mg/kg TOCP showed slight fragmentation of nerve fibers H&E 400X.

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التأثير العصبي لثلاثة أنواع من المركبات الفسفورية العضوية في الدجاج البالغ 1. التسمم الحاد

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المستخلص. أجريت هذه الدراسة لمعرفة أثار التسمم الحاد الذي تسببه ثلاثة أنواع من المركبات العضوية الفسفورية وهي (1.Diazinon, 2.Dichlorvos, 3.Tri-ortho- cresyl phosphate (TOCP)) في الجهاز العصبي للدجاج البالغ. لوحظ في هذا البحث كل من العلامات السريرية و الأفات العانية و التغيرات النسيجية التي أحدثتها المواد المذكورة في الجهاز العصبي. أستخدمت 24 دجاجة بالغة من عرق لوهمان بعمر 54 أسبوع ومعدل وزن حوالي 1.5kg. قسمت الطيور إلى أربعة مجاميع متساوية. ثلاثة مجاميع جرعت 0.8،320 و750 ملم/كغم من وزن الجسم من المواد الثلا ثة المذكورة على التوالي وأستخدمت المجموعة الرابعة كمجموعة سيطرة وتم التجريع عن طريق الفم مباشرةً. أحدث التجريع في هذه المواد علامات تسمم مسكارنية وشملت نفوش الريش، سيلان العاب، صعوبة التنفس وإسهال وكذلك أحدث التجريع علامات تسمم نيكوتينية متمثلة بالرعشة، تهدل الأجنحة، إضطرابات عصبية، شلل مع رقود كامل للطير على الأرض. إن فترة ظهور هذه العلامات وإختفائها وشدتها يعتمد على نوع المركب. فالمجموعة التي عوملت بالمركب رقم الأول أظهرت علامات سريرية أكثر شدة من الثاني بينما لم يحدث الثالث أيت علامات ظاهرة. ولم تظهر أيت تغيرات عيانية في الدماغ، الحبل الشوكي والعصب الوركي في المجاميع المعاملة. أما بالنسبة للتغيرات النسيجية فقد أظهرت المجاميع المعاملة وجود تفجي في المحاور العصبية للحبل الشوكي والعصب الوركي بسبب تنكس المحاور العصبية. وكانت هذه التغيرات أكثر شدة في المجموعة المعاملة بالمركب الأول وتليها المجموعة المعاملة بالمركب الثاني والثالث على التوالي وأظهرت النتائج إن التغيرات النسيجية للتسمم العصبي الحاد لهذه المركبات كان أكثر شدة في العصب الوركي أكثر مما هو عليه في الحبل الشوكي أما الدماغ فلم تظهر عليه أيت تغيرات نسيجية في جميع المجاميع المعاملة بالمواد الثلاثة المستخدمة في هذه الدراسة.