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ULTRASTRUCTURAL STUDY OF SUB CHRONIC NINETY DAYS NEUROTOXICITY OF TOCP (TRI ORTHO CRESYL PHOSPHATE) OF SPINAL CORDOF ADULT HEN

K. Majeed Saleh[1], E. Hadi Aula[2], A. Abd Alsamad Methaq[3] and A. Al-Sereah Bahaa[3]
[1]*No. 55 Desborough Road, Hartford- Huntingdom, Cambridgeshire, PE 29 1 SN, England.*
[2 & 3]*Department of Pathology & Poultry Diseases, College of Veterinary Medicine, University of Basrah, Iraq*

Abstract

The study was done for 90 days to study the neurotoxicity of TOCP in adult hens. In 4 groups of 20 hens per group, untreated control with corn oil, 1.25, 2.5 and 5 mg/kg of TOCP in corn oil. Then, electron microscopic examination was done with presence of higher number of degenerate myelinated nerve fibers in white matter then in spinal cord of acute neurotoxicity of TOCP. Degenerated myelinated nerve fiber were characterized by presence of vacuolation of myelin, spheroid body formation of degenerate myelin and ovoid lamellated bodies of degenerate myelin also presence of dark stained degenerate lamellated mitochondria. Occasionally, oligodendrocyte showed showed evidence of autophagocytosis of degenerate myelin.

Key words: Spinal cord, Sub-chronic, Electron Microscope and Hen.

1. Introduction

Degenerative pattern in the chicken central nervous system induced by ingestion of the organophosphorus delayed neurotoxin TOTP, a silver impregnation study was studied by (Abou Donia, 1979). Abou Donia, *et al*., 1979, studied delayed neurotoxicity of o-Ethyl-o-4-nitrophenyl phosphonothiate toxic effect of a single oral dose on nervous system of hen. Abou Donia, *et al*., 1983, studied the neurotoxicity of TOCP in adult hen reported changes in the synaptic vesicles and bontons. Al Sereah and Majeed, 2014, studied the absorbtion of TOCP through the skin of hens and its neurotoxic effects. Barrett and Oehme, 1994,studied biochemical, neuropathological and behavioral changes in hens induced by acute exposure of TOCP. Bischoff, 1970, studied delayed neurotoxicity of o-Ethyl-o-4-nitrophenyl phosphonothiate sub-chronic (90 days) oral administration in hen. Duke Tanaka and Steven, 1989, studied the mechanism of organophosphorus ester induced delayed neurotoxicity type I and type II. Gless and White, 1961, did neurotoxicity of TOCP of swine. Gupta *et al*., 2000, studied delayed neurotoxicity of phenyl phosphnothioate ester in hen. Itoh *et al*., 1984, studied the changes in the neurofilament of spinal cord. Majeed *et al*.,2014, studied the acute delayed neurotoxicity of TOCP in cat. Majeed *et al*.,2014, did a study on delayed neurotoxicity of organophosphorus for 35 days using single dose of 400 mg/kg of body weight in hen. Mohamed *et al*., 1990 did neurotoxicity on two organophosphorus ester flame retardants in hen and reported biochemical changes. Nanda and Tapaswi, 1995, reported changes in the CNS and PNS using di-isopropyl phosphorofluoridate in adult hen. Sprague *et al*.,

Corresponding author*: **K. Majeed Saleh
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1981, studied of acute delayed neurotoxicity of tri ortho cresyl phosphate (TOCP) of spinal cord light microscope of adult hen. Xiu Lan Zhaoa *et al*., 2006 studied the ultrastructural study of pineal gland in ageing sprague dawley rat.

2. Materials and Methods

The present study consist of 80 adult hen divided into 4 groups of 20 each as untreated control with corn oil dosed daily for 90 days also treated group as 1.25, 2.5 and 5 mg/kg in corn oil of TOCP daily for 90 days. Hens were sacrificed at the end of study, samples of spinal cord were fixed in special fixation (glutaraldehyde) then resin blocks were made and cut at 1ug by ultra microtome, then slide of thin sections were made and stained with toluidine blue for the selection of right areas for electron microscope, then Copper grids were done and stained with uranyl lead acetate and to be examine by Electron microscope.

3. Results

Electron microscope study showed high number of degenerate myelinated nerve fibers then in acute nurotox of TOCP. Degenerate myelinated nerve fibers were only seen in the white matter and characterized by degenerate myelin with vacuolation, spheroid bodies formation, lamellated gegenerate myelin, also presence of dark stained degenerate lamellated mitochondria. Oligodendrocyte with evidence of autophagocytosis characterized by the presence of degenerate myelin in their cytoplasm.

Fig - 1: Spinal cord, mitochondri with two patchy dense material in its matrix (EM 15000X)

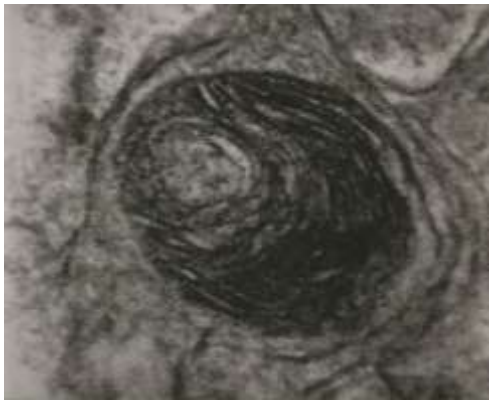

Fig - 2: Spinal cord, synaptic process replaced by multi lamellar body, note glia processes at the periphery (EM 21000X)

Fig - 3: Spinal cord, pre-synaptic process with dark stained multi lamellar bodies (EM 15000X)

Fig - 4: Spinal cord with vesicular degeneration, adjacent to apparently normal mitochondria (EM 15000X)

Fig - 5: Spinal cord, axon terminal with dark stained multi lamellar inclusion bodies (EM 15000X)

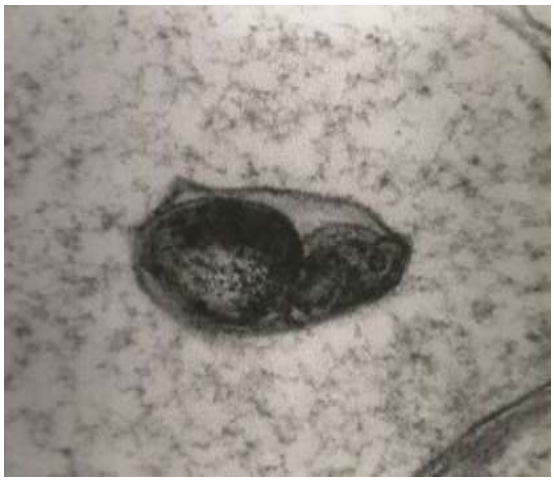

Fig - 6: Spinal cord, dendrite with layered lamellated dark stained body (EM 20000X)

Fig - 7: Spinal cord, dendrite with multi vesicular dark stained body (EM 20000X)

Fig - 8: Spinal cord, axon terminate with dark stained granulated/lamellar bodies (EM 20000X)

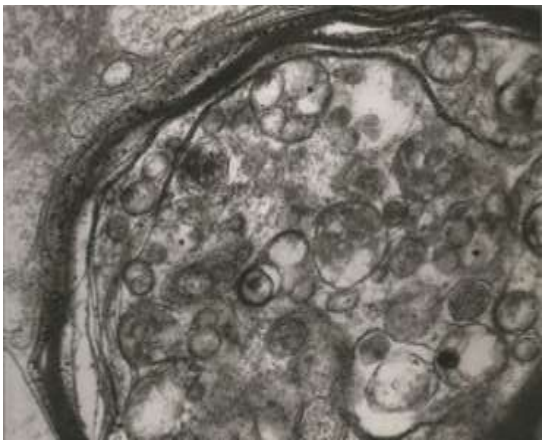

Fig 9: spinal cord,myelinated nerve fibers, with dark stained degenerate mitochondria in axoplasm (EM 15000X)

Fig 10: spinal cord,pre-synaptic process, note vesicular stages of degenerate mitochondria (EM 15000X)

4. Discussion

Neurotoxicity on TOTP as acute delayed neurotoxicity on CNS including spinal cord and found degeneration in nerve fibers was done by Adou Donia, 1979. The present study was on TOCP in adult hen for 90 days by oral administration daily in showed ultra structural changes in myelinated nerve fiber as degenerate myelin associated with proliferation of neurofilament and dark stained degenerate mitochondria in axoplasm. Abou Donia and Doyle Graham, 1979, studied toxic effect of single oral dose on the nervous system of hens and reported changes CNS and PNS. The present study was done for 90 days by daily dose of TOCP in adult hen and found electron microscopic changes in spinal cord as degenerate myelin and associated with dark stained degenerate lamellated mitochondria of the myelinated nerves fibers. Abou Donia *et al*., 1983 reported the changes in the vesicles and boutons of axosomatic synapses. The present study agreed about the changes in the synapses of the spinal cord but added presence of dark stained degenerate lamellated mitochondria in synapses and axoplasm of myelinated nerve fibers. Al Sereah and Majeed, 2014, studied the neurotoxicity of TOCP in hens by topical application on the skin and found the peripheral and central system (spinal cord). The present study was done on neurotoxicity of TOCP by 90 days and found electron microscopic changes as degenerate myelin associated with increase neurofilament and degenerate mitochondria in axoplasm. Barrett and Oehme, 1994, investigated the biochemical, neuropathological changes induced by acute exposure of TOCP in adult hen in central and peripheral nerve fibers. The present study was done on 90 days TOCP neuroxicity in adult hen and found electron microscopic changes in myelinated nerve fibers and mitochondria which could be incomparable with the above study. Bischoff, 1970, reported neuropathological changes in the spinal cord of 90 days by daily administration , the present study also for 90 days by daily administration of TOCP and found electron microscopic neuropathologic changes characterized by degenerate myelin, increase neurofilament with dark stained degenerate mitochondria in axoplasm of the above nerve fibers. Duke Tanaka and Steven, 1989, reported ultrastructural changes in the spinal cord as swollen axon with moderate amount of smooth endoplasmic reticulum and accumulation of mitochondria and neurofilament. The present study was done in adult hen for 90 days by daily doses and found changes in myelin sheath, increase neurofilaments with degenerate mitochondria in axoplasm of myelinated nerve fibers. Glees and White, 1961, did the acute neurotoxicity study of TOCP on swine and reported that they can be very good model for neurotoxicity and only reported changes in acetylcholine estrases. The present study was done for 90 day in adult hen and nerve fibers and mitochondria of axoplasm and considered adult hen as model for neurotoxicity. Gupta *et al*., 2000, reported histological changes in spinal cord and peripheral nervous after single dose. The present study was done on spinal cord by daily doses for 90 days in adult hen and found electron microscopic changes in myelinated nerve fibers as degeneration myelin and dark stained degenerate mitochondria in axoplasm of the nerve fibers. Itoh *et al*., 1984, did the acute neurotoxicity of TOCP and reported changes in neurofilament of spinal cord. The present study did 90 days neurotoxicity of TOCP by daily doses and found changes in myelin sheath, neurofilament and mitochondria of axoplasm of above nerve fibers. Majeed *et al*., 2014, studied acute neurotoxicity of TOCP in cat and reported clinical neuropathological signs which could be correlated with histpathological changes in PNS and CNS. The present study was done in adult hen for 90 day by daily doses and reported ultra structural changes in myelin sheath and mitochondria degeneration in axoplasm of the above nerve fibers. Majeed *et al*., 2014 observed the changes in the spinal cords and cerebullum, the present study was done on 90 days of TOCP using daily doses and found changes in myelin sheath and mitochondria in axoplasm of myelinated nerve fiber. Mohamed *et al*., 1990, induced neurotoxicity by two organophosphorus compounds on adult hen observing changes for 6

weeks. The present study was done on adult hens with TOCP for 90 days by daily administration and found electron microscopic changes in myelinated nerve fibers and mitochondria of axoplasm of treated hens. Nanda and Tapaswi, 1995, found changes in the CNS and PNS mostly biochemical. The present study was done on 90 days neurotoxicity of TOCP by daily treatment and found changes in spinal cord myelinated nerve fibers associated with degenerate lamellated mitochondria of axoplasm. Sprague *et al*., 1981, did acute delayed neurotoxicity of spinal cord of adult hens treated with TOCP (triorthocresylphosphate) as positive control for organophosphorus, histopathology of light microscopy of Toluidine blue stains showed occasional nerve fibers with partial demyelination also nerve fibers with clumps or masses of degenerate myelin. The present paper studied the electron microscopic changes in the mitochondria of the nervous system of adult hen due to neurotoxicity of TOCP in acute study. Xiu Lan Zhaoa *et al*., 2006, reported the electron microscopic study was done on young and old rats males and females showed presence of synapsis with dark core vesicles and numerous number of mitochondria, the presence of synapsis indicate electro chemical activity with possibility of active neurotransmitters. The present research did electron microscopic study on changes in mitochondria from various areas by neurotoxicity of TOCP in acute study. The result electron microscopy of sciatic nerve showed degeneration of myelin in myelinated nerve fibers characterized by vacculation of myelin, clumping of myelin, spheroid body formation of myelin, lamellated body of degenerate myelin, exoplasm showed increased of neurofilament also present of dark stained lamellated degenerate mitochondria. The present study was concentrated on electron microscopic changes in mitochondria of central nervous system induced by neurotoxicity of TOCP in adult hen.

5. Conclusion

Electron microscopic study gave fine information about the changes in different part of the spinal cord.

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