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The Histopathological Study of Brain of *Catla catla* Exposed to Dimethoate

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Abstract : The histopathological changes were evaluated in the brain tissue of Indian major carp, *Catla catla* after chronic exposure to an organophosphate pesticide, the dimethoate. The experimental group was exposed to sublethal dose of dimethoate (0.001 ppm) for four weeks. The different regions of brain were sectioned at 5-12 µ thick by paraffin embedding process. For the study of histology of nerve tissue silver impregnation technique was employed while for routine histology, the sections were stained with hematoxyline counter stained with eosin. The nerve cells showed necrosis, vacuolation, loss of cytoplasm and Nissl's bodies. The nerve bundles become loose and show dispersal of nerve fibrils in all parts of the brain. The severe damage was noticed in the optic tectum but less damage was noticed in the organelles of the Purkinje cells and also in the granular layer of cerebellum. The necrosis in axons of Mauthner cells in medullary region was prominent. The present study reveals that dimethoate even in 0.001concentraion is highly toxic to brain of the carp fingerlings and may lead to subsequent killing of fish.

Introduction

Dimethoate ($C_5H_{12}NO_3PS_2$) Tech. 95% is an organophosphate insecticide in EPA toxicity is class-II. The nerves are broadly been classified as cholinergic and adrenergic on the basis of neurotransmitters. It is unanimously accepted that AChE enzyme is present at the cholinergic synapse to deactivate the acetycholine neurotransmitter secreted at the cholinergic synapse. AChE is mainly activated by organophosphate, carbamate and some neurotoxic ligands as they pass through the P-site and phosphorelate the catalytic serine in the A-site (Johnson *et al.*, 2003). The cholinergic deficit is believed to be the main cause of cognitive decline noticed in Alzheimer's disease (AD) in human beings. According to Inestrosa and Colombres (2005), the only consistent treatment for AD symptoms has been controlled by the cholinesterase inhibitors. Inhibition of AChE results impairment of nervous tissue, respiratory and muscular functions in experimental animals as suggested by Hsssanein (2005).

Dimethoate reaches to water bodies through runoff during rainy season and damage to aquatic fauna including fishes. It is highly soluble in water. Dimethoate inhibits enzyme AChE activity and result in accumulation of ACh resulting in continuous transmission of impulse at the cholinergic synapse resulting death of the tissue in fishes (Bashamohideen & Sailbala,1989 and Kumar and Tembhre, 2010). Cardoso *et al.* (1996), Metcafe (1998), Balres (1999) and Cengiz *et al.* (2001) calculated maximal percent suppression and percent recovery of AChE activity in fishes and according to these authors the maximum activity is found in the brain and lowest in the intestine. Satydevan *et al.* (1993) and Gaur and Kumar (1993) studied AChE activity and enzyme kinetics in the brain and heart of common carp, *Cyprinus carpio* and *Channa punctatus* subjected to sublethal exposure to diamethote. According to these authors, diamethote is competitive in nature. Inhibitory kinetic study of AChE is very useful for monitoring the pesticide toxicity as suggested by Tembhre and Kumar (1994). Adams (1990), Oliveira *et al. (*2005) and Lionetto *et al.* (2005) suggested that AChE is a biomarker in environmental biomonitoring in tropical aquatic ecosystems. Parveen and Kumar (2005) described the biochemical estimation in the heart both in normal and pathological condition.

Begum and Vijayaraghhvan (1995) studied in vivo effect of dimethoate in the liver tissue of freshwater fish, *Clarias batrachus and* found that 65 mg/L for 96 h is quite toxic. Srivastava and Singh (2001) found that 17.9 mg/L for 96 h is toxic to *Channa punctatus*. Pandey *et al.* (2009) noticed low LC_{50} for 24, 48, 72 and 96 h dimethoate exposure was recorded inhibition of AChE as 3.38, 3.23, 3.08 and 2.98 µ/L respectively in *Heteropneustes fossilis*.

The behavioral changes have been reported due to dimethoate by almost all workers in this field. They found copious mucous secretion, reduced ability to maintain normal posture and balance with increasing exposure time (Kumar and Singh, 2000; Ram *et al.*, 2001; Bonita, 2004; De Mel *et al.*, 2005; Velmurugan *et al.*, 2007; Pande *et al.*, 2009; Singh *et al.*, 2010).

Some specific studies on histopathological effects of pesticides on the brain of fish were conducted by Satyadevan *et al.*(1993). Kumar *et al.* (1993) and Tembhre and Kumar (1994) studied effects of various

concentrations of organophoshate pesticides on various organs of *Cyprinus carpio* and reported the vacuolation in the cholinergic nerve cells and degeneration of Nissl's bodies and loosening of nerve bundle in various organs of fish.

Materials and Methods

A common major carp, *Catla catla* (length 3" to 4" and weight 10 $\pm$ 2 gm) were collected from Govt. Patra Fish Farm, Bhopal. After treatment with $KMNO_4$ for 2 minutes the specimens were acclimatization in glass aquaria (size 18"× 12"× 9"; capacity 25 lt.) for a week. Water was continuously aerated. The fingerlings were feed regularly with Shalimar fish food. Rogor 30% EC manufactured by Anil Products Limited, New Delhi was also used. Long term experiment was conducted on the fish for 30 days to study chronic toxicity of the pesticide. The LC_{50} (96 h) for dimethoate was determined by renewal bioassay test. The fishes were divided into two groups in glass aquaria. Ten fish were used for each group.

Group–1 was exposed to 1/5 of LC_{50} i.e. lowest sublethal concentration of pesticide.

Groups–2 was maintained in pesticide free water to serve as control.

Both the experimental and control fishes were sacrificed after 30 days. Brain was taken out from both groups, tissue was washed and cleaned in normal saline and fixed in Bouins solution and 10% formalin. The blocks were prepared according to paraffin embedding process. The sections were cut 5 to 12 µ (micron) thick. The sections were stained with haematoxylin counter stained with eosin and silver impregnation method. The microphotography was taken with the help of Olympus P.A. 6 equipment.

Results and Discussion

The behavioural changes were noticed in brain of fingerlings of *Catla catla* with the chronic exposure to sub-lethal concentration (0.001 ppm) of dimethoate. The fingerlings showed erratic swimming, increased surfacing and loss of balance and the fingerlings persistently loose balance with increasing exposure. They showed profuse secretion of mucous and decreased rate of opercular movements. Further, during 1st week, the opercular beats increased but later the opercular beats gradually decreased during 2nd, 3rd and 4th week of exposure. It is argued that decreased rate of opercular movements helps the fish in reducing absorption of poison and increased the period of their survival in such toxic environment. Regarding toxic behavior of organophosphate pesticide is in confirmation to the observations of earlier investigators (Kulshrestha and Jauher, 1986; Richmonds and Dutta, 1989; Cardoso *et al.*, 1996; Kumar *et al.*,2000; Rao *et al.*, 2005; Mahira and Kumar 2005; Singh *et al*., 2010; Kumar and Tembhre, 2010; Ricardo *et al*., 2011; and Moitra *et al.*, 2012).

As regards the fish brain, the majority of workers did not find any damaging effect of pesticide on the morphology of fish brain and they also did not find any impairment in the choroid plexi (Lal, 1969; Srivastava and Dubey, 1973; Srivstava and Kumar 1975; Bhattacharya and Mukherjee, 1978; Sastry and Sharma 1981; Kulshreshtha and Arora, 1984; Sahai and Thakur, 1989). In contrast to the observations of the above referred authors, the present investigation shows that color of brain became pink to yellow. This is due to hypoxic condition resulting effecting hemoglobin deformation on account of pesticide. The network of blood vessels was also not visible. This indicates that the pesticide penetrated into blood vessels and disrupted the normal structure of blood vessels.

In telencephalon, necrosis of nerve cells has been noticed. The gap of ypsiliformes has become large in size and the outer lining of the sulcus also showed disorganization (Fig. 1). Increased numbers of vacuoles are seen, less cytoplasm has been noticed and in some of these cells there is a gap between the cell membrane and cytoplasm. The nerve fibrils in anterior commissure become very less in number and are found in dispersed manner (Fig. 2). Neuropathological findings based upon silver impregnation noticed high degree of vacuolization in nerve cell and perikoryon became impaired at several places. The nuclei became acentric in position and there is loss of cytoplasm in nerve cells (Fig. 3). The axons and dendrites too show degeneration. The nerve bundles in both telencephalon and diencephalon showed degeneration. The optic tectum and cerebellum also showed severe necrosis. Metheissen and Robert (1982) and Singh (1985) reported encephalitis in optic tectum of *Tilapia rendalli* after exposure to endosulfan . Encephalitis, vacuolation and dispersed nerve fibres are seen in rest of layers of optic tectum (Fig. 4). Nerve fibres joining optic tectum to torus longitudinalis (Singh and Khanna 1970) are found less in number and show clear signs of degeneration (Fig. 4). Partial necrosis has been seen in axons of Mauthner's cells in medullary region, degenerated and dispersed nerve fibres are distinguished feature of the present study.

The III ventricle in the diencephalon losses its shape and became shrunken. Vacuolation were noticed in the neurosecretary cells. A great degree of vacuolation and necrosis are noticed in granular and molecular layers of cerebelli (Fig. 5). Cerebellar cortex is badly affected in molecular layer, large vacuoles,

clumped nerve fibres are very clear (Fig. 6). Purkinje's cell layer and granular layer are not much affected. In the medullary region, columns of nerve fibres are found to be degenerated. Nerve fibres are lying dispersed thus large gaps are seen among nerve fibres (Fig. 7). Cytoplasmic congestion has been noticed in the cells of medullary region (Fig. 8). The present findings are in conformity to the observations to Ricardo *et al*. (2011) and Xing *et al*. (2012).

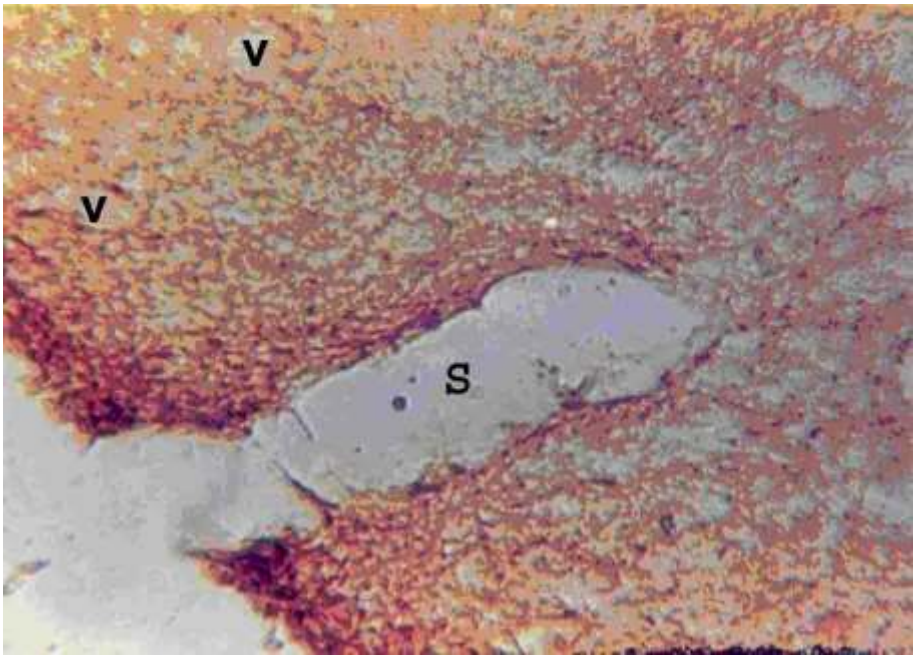

Fig. 1. Photomicrograph of T. S. of telencephalon of *Catla catla* showing large gap of suculus (S) and vacuolization (V) after chronic exposure to dimethoate (0.001 ppm). X 400

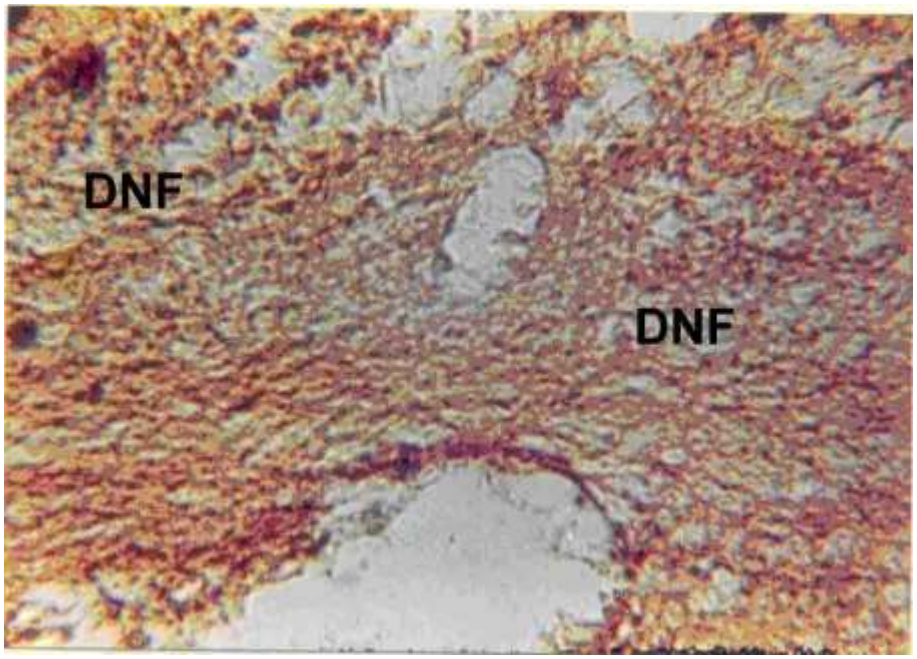

Fig. 2. Photomicrograph of T. S. of telencephalon of *Catla catla* showing meager and dispersed nerve fibrils(DNF) in anterior commissure after chronic exposure to dimethoate (0.001 ppm). X 400

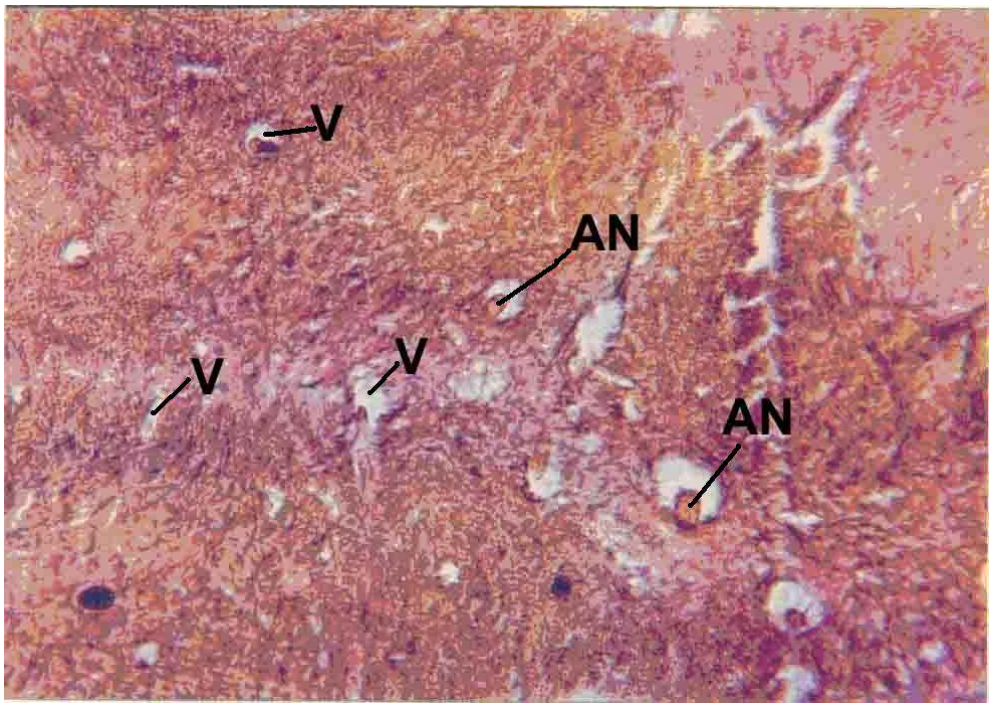

Fig. 3. Photomicrograph of T. S. of diencephalon of *Catla catla* showing nerve cells with accentric nuclei (AN) and vacuolization (V) after chronic exposure to dimethoate (0.001 ppm). X 100

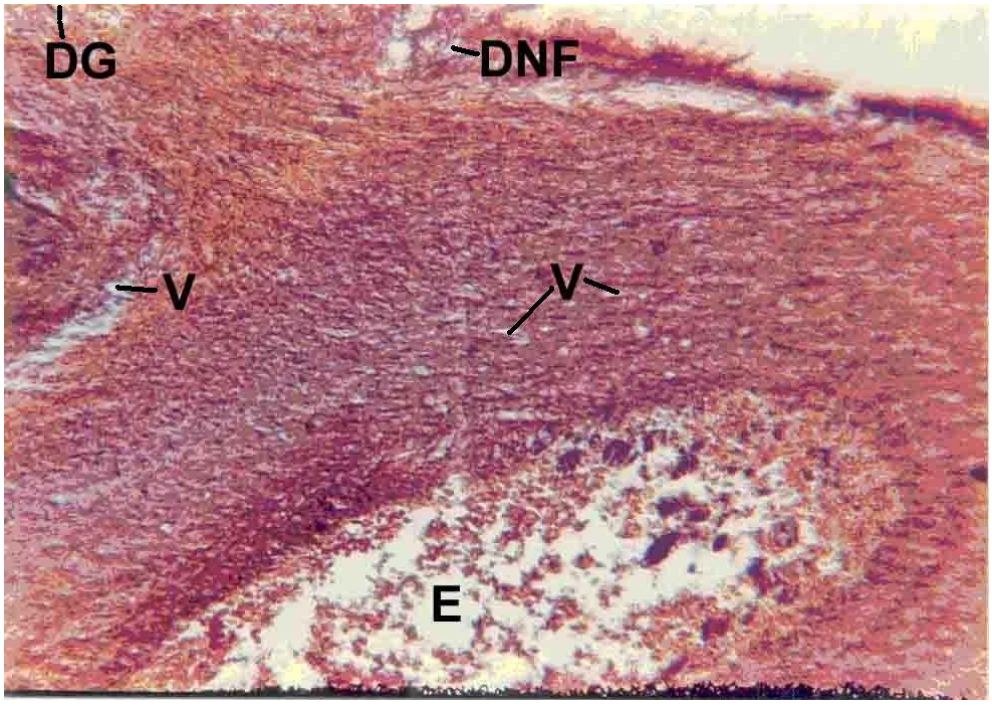

Fig. 4. Photomicrograph of T. S. of mid brain of *Catla catla* showing degeneration (DG), encephalitis (E), vacuolization (V), dispersed nerve fibres (DNF) in various layers of optic tectum after chronic exposure to dimethoate (0.001 ppm). X 100

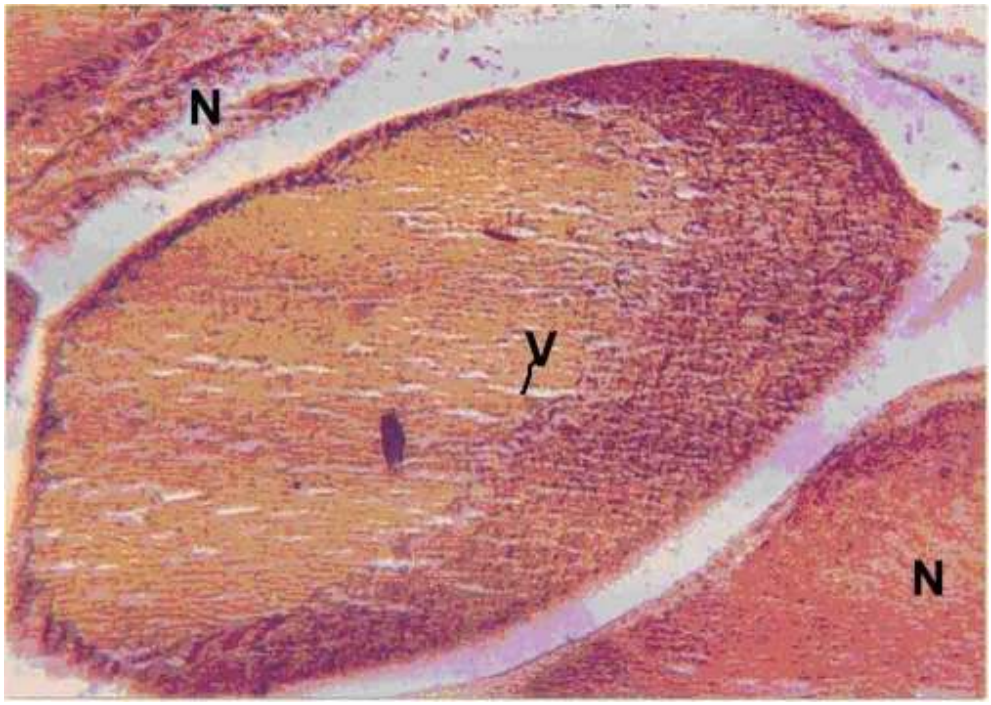

Fig 5. Photomicrograph of T. S. of mid brain of *Catla catla* showing vacuolization (V) and necrosis (N) in granular and molecular layer of valvula cerebella after chronic exposure to dimethoate (0.001 ppm). X 100

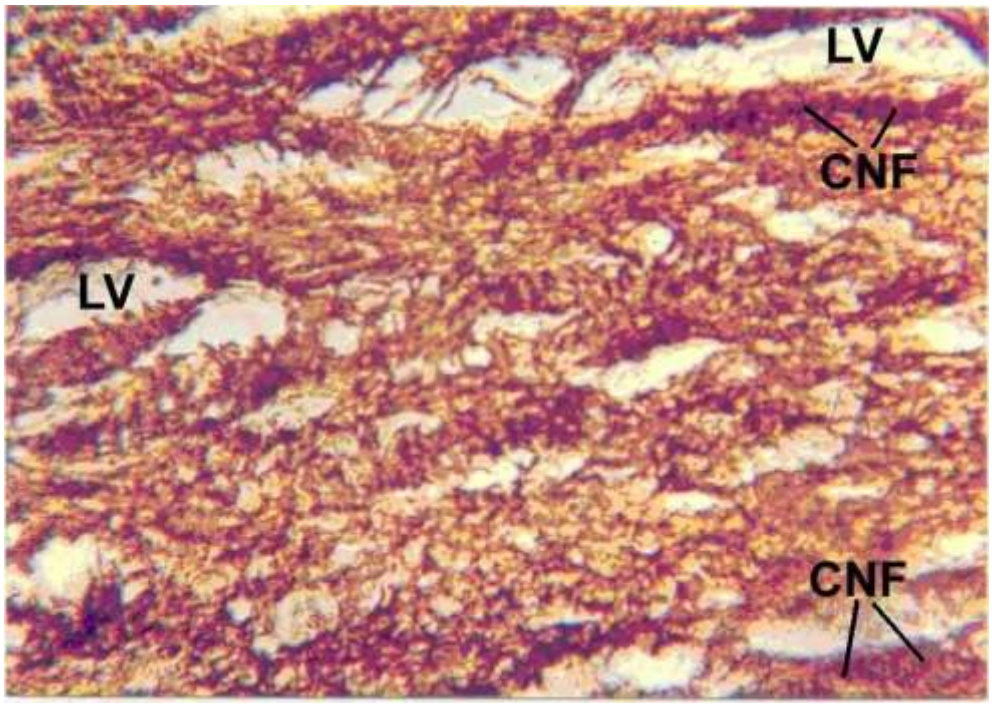

Fig. 6. Photomicrograph of T. S. of through cerebellar cortex of *Catla catla* showing large vacuoles (LV), clumped nerve fibres (CNF) in molecular layer after chronic exposure to dimethoate (0.001 ppm). X 400

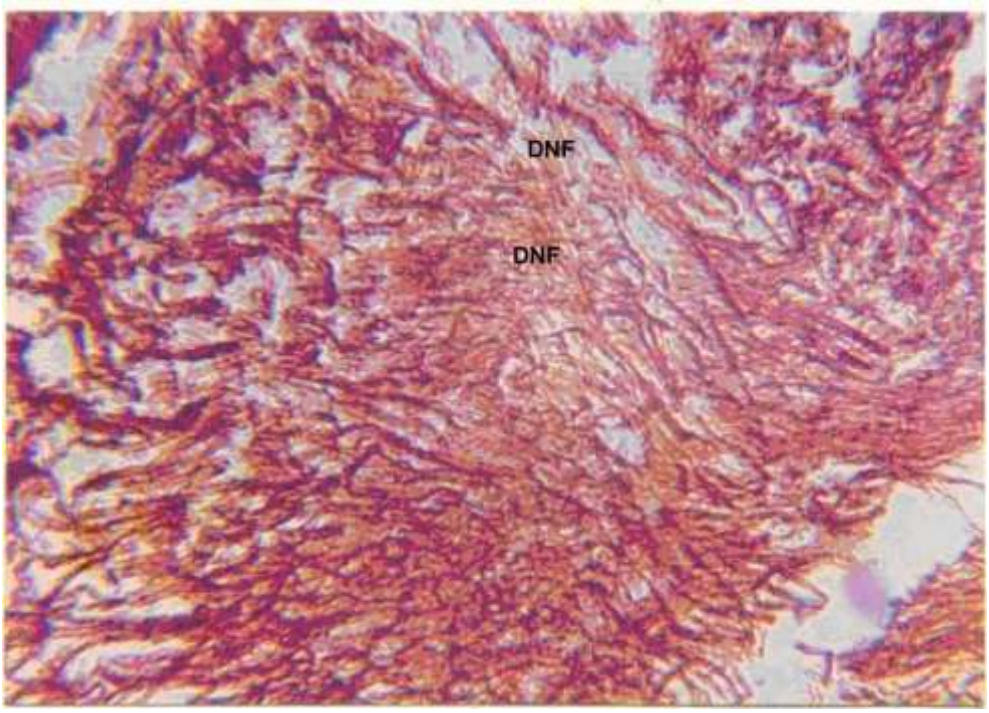

Fig. 7. Photomicrograph of T. S. of medullary region of *Catla catla* showing degenerated nerve fibres (DNF) after chronic exposure to dimethoate (0.001 ppm). X 400

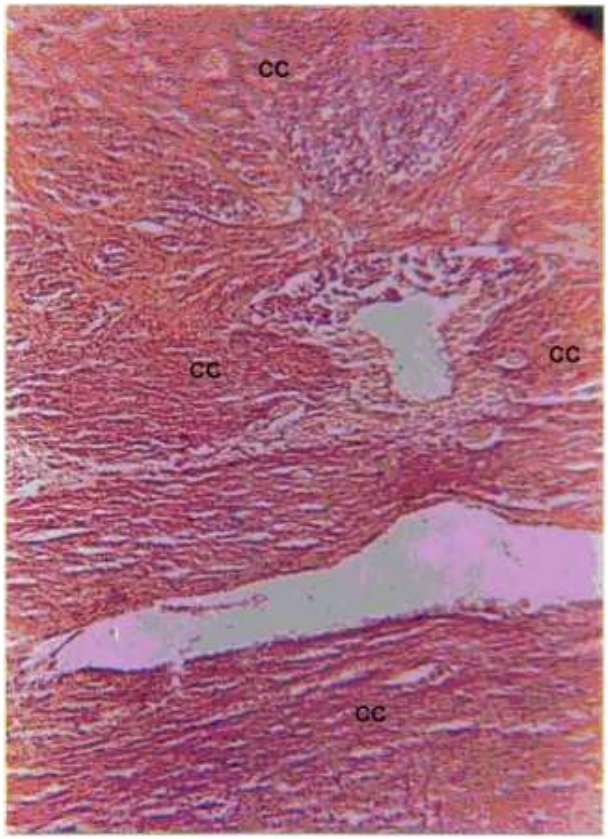

Fig. 8. Photomicrograph of T. S. of medullary region of *Catla catla* showing cytoplasmic conjestion (CC) after chronic exposure to dimethoate (0.001 ppm). X 400

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