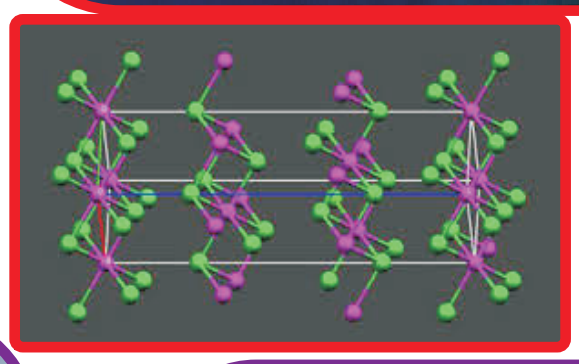
EFFECT OF CADMIUM ON THE ULTRA STRUCTURE OF SPLEEN OF TILAPIA MOSSAMBICA (PETERS)

Abstract:-

The present work deals with the cadmium induced ultra structural changes found in the spleen of Tilapia mossambica (Peters). Fishes were exposed to a sub lethal concentration of 8 ppm of CdCl2 for 6 days and 22 days. Treatment induced changes differ from one cell type to the other. Some of which are common to all cell types include vacuolation of cytoplasm, appearance of vesicles within the cytoplasm, increased endoplasmic reticulum, vacuolation of mitochondria and deposition of electron dense material.



Keywords:

Tilapia mossambica, spleen, CdCl2, ultrastructural changes.

Meenakshi Sundaresan

Head & Associate Professor , Department of Zoology, D. G. Ruparel College, Senapati Bapat Marg, Mahim.Mumbai. Maharashtra, India.

INTRODUCTION

Heavy metals are known hazardous pollutants which are toxic to living organisms. Environmental exposure to heavy metals due to anthropogenic activity can result in greatly increased levels in the environment resulting in diseases in humans and other animals. Their accumulation in aquatic organisms, including fish, needs continuous monitoring and surveillance owing to the biomagnifying potential of these metals in the food chain (Ishaq *et al* 2011; Kumar Babu *et al*., 2009). A lot of histological and histochemical work has been done in fishes regarding pollution effects on various tissue organs like alimentary tract, liver, gills, kidney, etc. (Sundaresan, 2014a; Okacha, 2011; Puneet and Anu, 2010; Sundaresan *et al*., 2009; Van Dyk *et al*., 2009; Uttam Kr Sen *et al*., 2008; Loganathan *et al*., 2006; Thopon *et al*., 2004; 2003). But few have taken up spleen for study of pollution effects (Sundaresan 2014b; Ali Louei *et al.*, 2013; Basim 2008). This study was undertaken to study the effects of $CdCl_2$ on spleen of *Tilapia mossambica (Peters).*

MATERIALS & METHODS:

Live Tilapia fish were obtained from Masunda Lake in Thane district in Maharashtra, India and were kept for a fortnight for laboratory acclimatization. Five sets of ten fishes were treated with sub lethal concentrations of 8 ppm of CdCl2 for 6 and 22 days under standard APHA (1985) conditions based on LC_{50} studies. The spleen of fish (control & treated with 8 ppm $CdCl_2$ for 6 days and 22 days) were fixed in 3 % glutaraldehyde for 30 minutes at 4ºC and was processed for electron microscopy. Ultra thin sections were cut on the LKB ultramicrotome and picked up on G-200 copper grids. They were stained for 1 hour with uranyl acetate and counter stained with lead citrate. Grids were scanned under a Ziess EM 109 electron microscope and JEM joel 100 'S' electron microscope.

OBSERVATIONS:

Electron micrographs of spleen in treated fish shows changes at the ultra structural level. Though changes differ from one cell type to the other, there are certain changes which are observed more or less uniformly in all cells (Fig. 1). Such changes include vacuolation of cytoplasm, appearance of vesicles with granular material, increase in the extent of endoplasmic reticulum, vacuolation of mitochondria and the deposition of electron dense material in comparison to the control (Fig. 2).

Various changes noted in individual cell types are as under:

Endothelial cells: The endothelial cells lining blood capillaries in treated fish show appearance of cytoplasmic vacuoles. When compared to endothelial cell of control spleen, there is increase in the number of microfibrils and cytoplasmic granules (Fig. 3). The margin of endothelial cells has been found to get pinched off. Several of such pinched off vesicles of varying sizes and shapes can be seen within the sinuses facing the cell surfaces of endothelial cells (Fig. 4, 5) which is not observed in control spleen (Fig. 14) as described by Sundaresan (2014c).

The tubules of endoplasmic reticulum have been found to get enlarged to a great extent. These tubules often get dilated enormously to produce vesicles of varying sizes and shapes. The vesicles indicate the presence of some dense material within (Fig. 6).The undilated, intact endoplasmic reticulum tubules that are around the nucleus are observed to be at right angles to the nuclear membrane. Unlike other cells, the nuclear membrane of endothelial cells remains fairly intact and the two membranes (outer and inner) are not separated. So perinuclear space which is distinctly visible in other cells is not that evident in these cells (Photographs nos. 5,6).The chromatin material that is within the nucleus shows signs of pycnotic condition at various sites of nuclear region. The appearance of dense fibrils which are wavy in outline is yet another characteristic feature (Fig. 6).

In extreme conditions, the cells are seen with the deposits of electron dense material. Such deposits are of different sizes and shapes and are not seen in endothelial cells of control spleen. Desmosomes appear darker and this seems to be mainly due to the presence of electron dense material within the intercellular spaces (Fig. 7).

RETICULOCYTES:

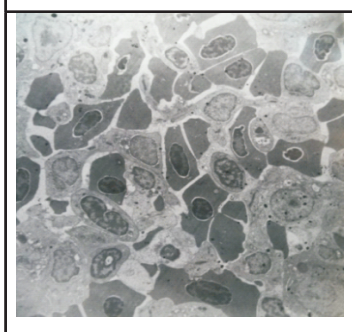
In normal spleen these are elongated cells that taper at the two extremities and show presence of large prominent nucleus. Cytoplasmic vacuolation and granules are seen in increased numbers (Fig. 1) with treatment of $CdCl_2$.

MEGAKARYOCYTES:

Megakaryocytes too show drastic changes with the treatment of cadmium chloride. These cells are often deshaped (Fig.7) as compared to oval or spherical shape of normal megakaryocytes. The

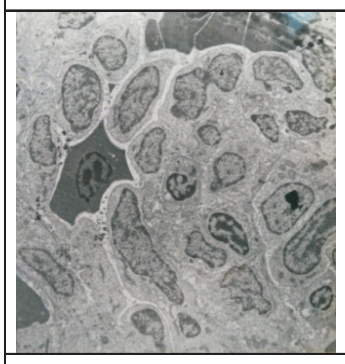
cytoplasm shows a high degree of vacuolation. The cytoplasmic granules are numerous and these are of coarse type. The endoplasmic reticulum tubules are dilated at various places.. Mitochondria are seen with vacuolation as compared to mitochondria of normal megakaryocytes. Presence of vesicles and appearance of pigment deposits are the other noticeable changes (Fig. 7).Shape of the nucleus too gets altered with the treatment. Often nucleus is seen with a constriction in the middle. Perinuclear space is also distinct.

Fig 1: EM . and diagrammatic sketch of Spleen showing different cell types, treated 8 ppm 6 days



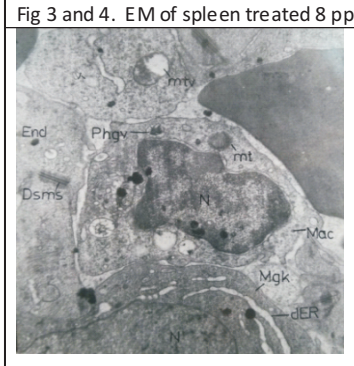
Key
End – Endothelium, Eo – Older Erythrocytes, Em – Mature Erythrocyte, E1 – Erthrocytic series, dsm – Desmosomes, dm – dense material, Mgk – Megakaryocyte, R – Reticulocyte, M – Monocyte, N - Neutrophil

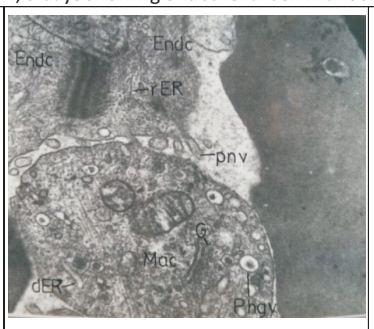
Fig 2 EM and diagrammatic sketch of spleen (control) showing various types of cells.



Key: E1 – erthrocytic stage, Eo – older erythrocyte, Enp – endothelial cell process, L – lymphocyte, N – neutrophil, Mgk – Megakaryocyte, M – Monocyte, R – Reticulocyte, Mc - Macrophage

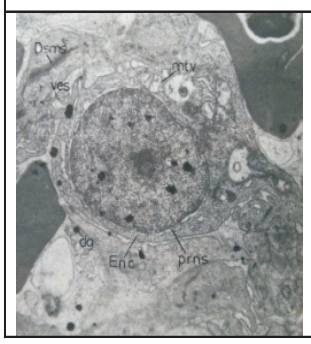
Fig 3 and 4. EM of spleen treated 8 ppm, 6 days showing endothelial cell with cell junction

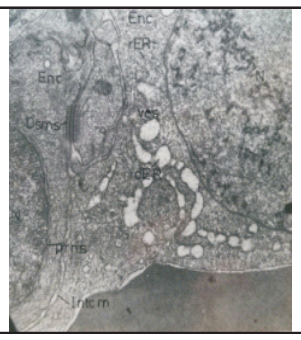




Phgv – phagocytic vesicle, N – nucleus, v – vesicle, Endc – endothelial cell, mt – mitochondria, Mac – macrophage. Mgk – megakaryocyte, dsms – desmosomes, G – golgi body, rER – rough endplasmic reticulum, pnv – pinched off vesicle, l – lumen, mtv – mitochondrial vacuolation, dER – dilated endoplasmic reticulum

Fig 5 and 6: EM of spleen treated 8 ppm, 6 days, 22 days showing endothelial cells





Key: rER – rough endoplasmic reticulum, N – nucleus, ves – vesicle, Enc – endothelial cell, Intcm – intercellular membrane, dg – dense granule, Dsms – desmosome, prns – perinuclear space, mtv – mitochondrial vacuolation, dER – dilated endoplasmic reticulum

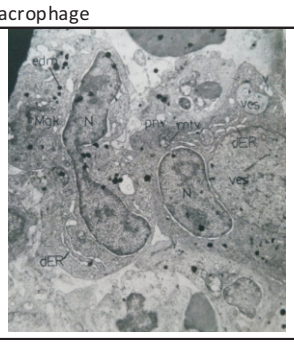
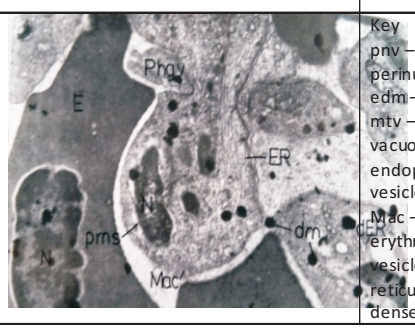
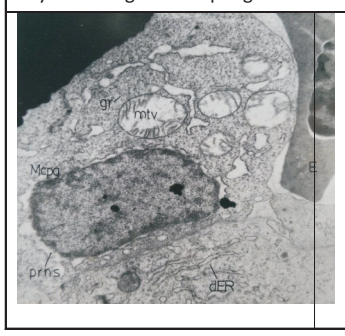
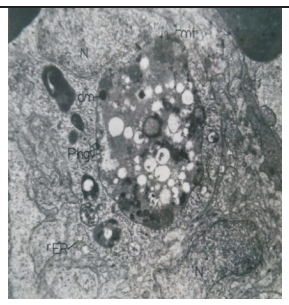
Fig 7: EM of Spleen, treated 8 ppm, 6 days showing Megakaryocyte and Macrophage	Fig 8: EM of spleen treated 8 ppm, 6 days showing Macrophage	
		Key pnv – pinched off vesicle, prns – perinuclear space, v – vacuoles, edm – electron dense material, mtv – mitochondrial vacuolation, dER – dilated endoplasmic reticulum, ves - vesicle Mac – Macrophage, E – erythrocyte, phgv – phagocytic vesicle, ER – endoplasmic reticulum, N – nucleus, dm – dense material
Fig 9: EM of spleen treated 8 ppm, 6 days showing 2 macrophages	Fig 10: EM of spleen treated 8 ppm, 22 days showing macrophage	Key
		Mcpg – macrophage, E – erythrocyte, mtv – mitochondrialvacuolation, dER – dilated endoplasmic reticulum, prns – peri nuclear space, Edm – electron dense material, dm – depository material, phgv – phagocytic vesicle, dsms – desmosomes, rER – rough endoplasmic reticulum, N – nucleus, cd – cell debris

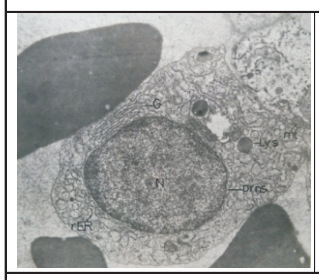
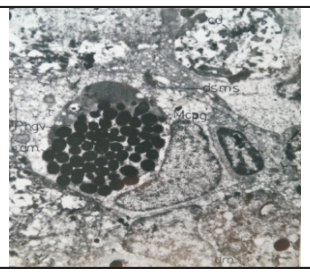
Fig 11 and 12: EM of spleen treated 8 ppm, 22 days showing macrophage		
		Key G – golgi body, Lys – lysosom e, prns – perinuclear space, N – neucleus, rER – rough endoplasmic reticulum, mt - mitochondia

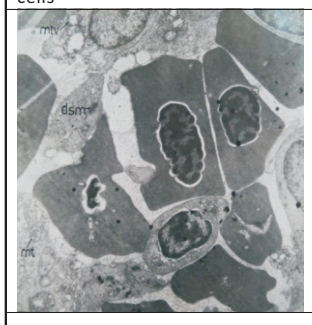
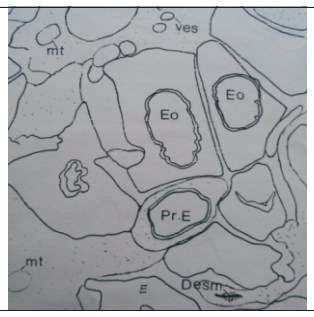
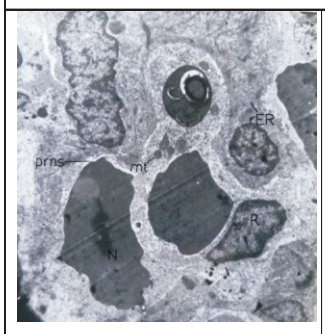
Fig 13: EM and diagrammatic sketch of spleen treated 8 ppm, 22 days showing blood capillary filled with blood cells		
		Key Eo – older erythrocytes, E – erythrocytes, prns – perinuclear space, PrE – pre erythrocyte, dsm – desmosomes, ves – vesicles, mt - mitochondria

Fig. 14E.M.and diagrammatic sketch of Spleen (control)showing blood vessel and the surrounding tissue.



Key
Cytp – cytoplasmic process, Dsm – Desmosomes, End – endothelial cell, Eo – older erythrocyte, L – Lumen, mt – mitochondria, N – Nucleus prns – perinuclear space, rER- rough endoplasmic reticulum, R – reticulocyte, mct – microtubules, phgV – phagocytic vesicle

MACROPHAGES:

Normally macrophages have an irregular shape and small eccentrically placed nucleus. Cytoplasm is full of granules and endoplasmic reticulum is seen along with oval shaped mitochondria. However, treatment with $CdCl_2$ affects them quite visibly. .The endoplasmic reticulum tubules are seen in a highly dilated condition. The cytoplasm is full of dilated vesicles. The membranous lining of the vesicles is granular and they are usuallyfilled with some dense material. Lysosomes are prominent. The cytoplasmic granules are of coarse type. Mitochondrial vacuolation is evident on a large scale (Fig. 8, 9).

In extreme cases the cells are seen with large vesicles filled with electron dense material. These cells have their endoplasmic tubules also filled with dense material. The presence of dense material within gives a fibrillar look to the tubules. Golgi is very prominent. The dilation of plasma membrane is yet another characteristic of such cells. Phagocytic vesicles are also seen very often (Fig. 10-12). Nuclear membrane is not clear but the perinuclear space is distinct.

ERYTHROCYTES AND ERYTHROCYTIC SERIES:

The treatment effects noted are the wavy nature of the nuclear margin, increase in the size of perinuclear space and the occurrence of electron dense depository material within the cytoplasm. Pre-erythrocytic cells wherever seen have cytoplasmic vacuoles. Perinuclear space is prominent. Depository material is also seen at several places in the cytoplasmic region (Fig.13). Erythrocytic cells of the control spleen can be seen in Fig. No. 2 and 14.

LEUCOCYTES AND LEUCOCYTIC SERIES:

The changes that are noted in these cells after the treatment are the appearance of cytoplasmic vacuoles and the large scale deposition of dense material. Mitochondria wherever seen are in vacuolated condition. (Fig No. 1). EM of spleen (control) showing various types of leucocytes is seen in Fig. 2.

RESULTS AND DISCUSSION:

Histological changes induced by cadmium chloride include hypochromic condition, necrosis and large scale deposition of hemosiderin. Similar changes have been reported by earlier workers too (Sundaresan, 2014b; Basim, 2008; Awari, 1991). The present study wherein the fish has been subjected to different periods of exposure to $CdCl_2$ treatment confirms the findings of earlier workers and these changes have been found to get aggravated with the increased period of exposure (Sundaresan, 2014b; Awari, 1991). Also extent of haemosiderin deposition has been found to be related to the period of exposure. Increased haemopoietic activity is yet another condition reported as an effect of treatment (Basim, 2008). The present study shows that increased haemopoiesis is evident only in the early stages of treatment. This may be probably an attempt to make good the effect of large scale destruction of erythrocytes. However the continued exposure the $CdCl_2$ seems to affect the haemopoietic tissue itself bringing down the production of blood cells.

The electron microscopic study of spleen shows that practically every type of splenic cell is affected by the treatment. The endothelial cells lining the blood vessels have been found to give out several of cytoplasmic vesicles. The condition of necrosis reported under light microscopic studies appears to be due to the large scale aggregation of such vesicles. Of the various effects noted, the most striking ones are the vacuolation of cytoplasm; separation of cells, formation of cytoplasmic vesicles, the accumulation of electron dense material and the vacuolation of mitochondria. Dilation of endoplasmic reticulum is observed in a highly pronounced state in the endothelial cells. All these changes represented in almost all cells of the spleen. These are however not reported in control (Sundaresan, 2014c).

The treated cells have been found to be full of electron dense materials which appear to be of two types – particles of cadmium salt used for treatment and haemosiderin that is formed owing to the destruction of erythrocytes. The electron micrographs of both these depository materials appear alike. They appear as masses of varying sizes and distinguishing the two types of materials poses a problem. However, it should be noted that particles of the treated materials are represented in all cells. These particles have more irregular outline. The haemosiderin bodies on the other hand are either seen within the phagocytic vesicles of macrophage or they occur as intercellular deposits. Such deposits are spherical in outline and are usually seen in aggregations.

Increased numbers of macrophages and megakaryocytes are seen prominently in the electron micrographs of treated fish. Some of the earlier workers who have carried out pollution studies have suggested the kidney and liver to be the main target organs (Gaikwad et al., 1990; Shareef Khalid *et al*., 1986). The present study however reveals spleen to be the main site of accumulation.

ACKNOWLEDGEMENTS

Author is grateful to Dr. S.V. Shanbhag for providing valuable guidance and encouragement for this research; The Director of CRI, Parel for providing laboratory facilities;Jaslok Hospital for electron microphotography and to Dr. Gayathri N. for reviewing the paper.

REFERENCES:

1.Ali Louei; Monfared, Sahar Hamoon Naward, Zahra Bakhteyari, Hajar Azizian and Sara Rahimi (2013). Histological changes of the lymphatic organs and white blood cell count following formaldehyde administration in the rainbow trout. Eur. J. of Expt. Bio., 3(3):572-575.
2.APHA(1985) 'Standard methods for the examination of water and waste water'. APHA/AWWA/WPCF, Washington D.C, USA.
3.Awari S.B. (1985) Toxic effects of cadmium on edible fish, Ambassis ranga (Hamilton Buchanan) M.sc. Thesis University of Bombay.
4.Awari S.B. (1991) Toxicity and depuration studies of cadmium on Ambassis ranga (Cuvier-Valenciennes) with reference to variation in environmental and some biochemical parameters. Ph.D. Thesis University of Bombay.
5.Basim M. Jasim (2008). Effects of prolonged exposure to cadmium on the haematopoietic organs in grass carp (Ctenopharyngodon idella, Cyprinidae). Bas .J. Vet. Res; 7(2): 108-120
6.KumarBabu D., Obaiah, J., SudhakarReddy P., Bhavani G. and Usha Rani A. (2009): Combined effect of copper, mercury and cadmium induced bioaccumulation in the selected tissues of two aquatic organisms . Aqua Biol; 24(2), 177-181.
7.Gaikwad S.A and Rege M.S.(1990) Effects of chronic exposure to pesticides, Thiodan 35 EC and phenyl mercuric acetate on the various tissues of Tilapia mossambica (Peters). Environ. Concern and Tissue Injury. Vol IV pgs.179-192.
8.Ishaq Eneji, Rufus Sha Ato and Annune P.A. (2011) Bioaccumulation of heavy metals in fish (Tilapia zilli and Clarias gariepinus) organs from River Benue, North Central Nigeria. Pak. J. Anal. Environ. Chem. 12 (1, 2): 25-31.
9.Loganathan K., Velmurugan B., Hongray Howrelia J., Selvanayagam M., Bhusan, Patanik B. (2006). Zinc induced histological changes in the brain and liver of Labeo rohita(Ham).Journal of Environmental biology 27:107-110.
10.Okacha R.C and Adedeji O.B. (2011).Overview of Cadmium Toxicity in fish. Journal of Applied Sciences Research 7(7):1195-1207.
11.Puneet kumar and Anu singh(2010) Cadmium Toxicity in fish: An overview. GERF Bulletin of Bio sciences 1(1):41-47
12.Shareef Khalid, Shareef Shakeela, Wagh S.B.(1986).Effect of sublethal dose of Suquin(R)(Quinalphos 25% w/w) on liver and kidney of 2 fresh water cyprinid fishes, Barbus ticto and Rasbora laniconicus (Ham).Environmental Biology, coastal Ecosystem pg 93-100.(C). The Academy of Environmental Biology, India.
13.Sundaresan Meenakshi (2014)a. Behavioural and histochemical changes induced by CdCl2 on alimentary tract of freshwater fish, Tilapia mossambica (Peters). Review of Research Journal 3(4):1-8
14.Sundaresan Meenakshi (2014)b Cadmium induced histological and histochemical changes in the spleen of fresh water fish,Tilapia mossambica(Peters). Indian Streams Research Journal, 4 (3):1-6

15.SundaresanMeenakshi (2014)c Ultrastructure of spleen in fresh water fish, Tilapia mossambica (Peters). European Academic research 2 (2): 2894 to 2908
16.Sundaresan Meenakshi and Shanbhag S.V. (2009). Effect of cadmium on the blood of Tilapia mossambica (Peters) J.Aqua. Biol.24:193-198
17.Thopon S., Pokethitiyookn P., Chalermwatk., Upatham E.S., Sahaphong S.(2004) Ultrastructural alterations in the liver and kidney of white seabass Lates calcarifer in acute and subchromic cadmium exposure. Environ. Toxicol.19:11-19
18.Thopon S., Kruatrachue M., Upatham E.S., Pokthitiyook K.P., Sahaphong S., Jaritkhuan S.(2003) Histopatological alteration of white seabass, Lates calcarifer in acute and sub-chronic cadmium exposure. Environ. poll 121:307-20.
19.Uttam kr Sen and Apurba Ratan Ghosh (2008). Histological and ultra microscopical alterations in the regions of gastro intestinal tracts of Anabas testudineus (Bloch) induced by Cadmium chloride.
20.Van Dyk J.C., Pieterse G.M., Van Vuren J.H. (2007) Histological changes in the liver of Oreochromis mossambicus (cichlidae) after exposure to cadmium & zinc. Ecotoxicology and environment safety. 66:432-40.