

Cadmium Toxicity in Kidney Ultrastructure of Freshwater Fish, *Oreochromis mossambicus* (Peters)

Meenakshi Sundaresan

Department of Zoology, D. G. Ruparel College, Mahim, Mumbai 400016, India
meenakshi.sundaresan[at]ruparel.edu

Abstract: *Kidney is an important organ – vital for excretion, osmoregulation and maintenance of homeostasis; various environmental pollutants like xenobiotics and heavy metals are known to affect its morphology. Fishes can thus act as heavy metal indicators and assess pollution in the aquatic environment. The present work deals with treatment of fishes with cadmium chloride (8ppm for 6days). The changes brought about by cadmium toxicity to the kidney of Oreochromis mossambicus (Peters) was studied using electron microscopy. Light microscopy and ultrastructure of kidney indicates that it consists of 5 distinct regions, namely, neck region, proximal region, central slender region, distal region and collecting tubule. Kidney of cadmium treated fishes show presence of electron dense material within the cytoplasm. Blocking of junctional complex, dilation and fragmentation of endoplasmic reticulum, wavy plasmalemma invaginations, large scale appearance of cytoplasmic vacuoles, occurrence of autophagic vesicles, increased lysosomes, vacuolation/fragmentation of mitochondria are the other changes seen.*

Keywords: Electron dense vesicles and vacuoles, dilated ER, mitochondrial vacuolation and fragmentation

1. Introduction

The careless disposal of heavy metals in the aquatic system is a cause of concern because of their toxicity and biomagnification. Cadmium is known for its non-corrosive nature and is widely used in manufacturing batteries, paints and dyes, fertilizers and also in the plastic industries. Cadmium release in the environment is steadily increasing due to anthropogenic activities causing pollution of soil and aquatic ecosystems. Biomagnification of Cadmium takes place at trophic levels and is found to be highest in algae [1]. It also accumulates in many aquatic organisms including fish which are a part of the aquatic food chain [2] [3] [4]. In the fish *Dicentrarchus labrax* and *Centropomus undecimalis*, Cadmium has been reported to induce histological and cytological changes to kidneys [5]. Cadmium is found to be teratogenic, embryo toxic, carcinogenic, nephrotoxic not only in fishes but humans too [6] [7]. It acts as a stressor affecting enzymes, which control all the biochemical reactions of the cell in particular and the organism as a whole. The present study has been done to evaluate the effects of cadmium chloride on the Ultrastructure of Kidney in *Oreochromis mossambicus* (Peters).

2. Materials and Methods

Live fish (3-5 inches) were obtained from Masunda lake in Thane district and were acclimatized for 2 weeks. They were fed on alternate days with live tubifex worms. During experimental exposure, to maintain the concentration of toxicant, test water was changed every 24 hours. The tanks were aerated with oil free air. Test water quality was evaluated employing standard methods [8].

In the present study, (2 sets) each with 15 fishes were maintained in 20 litres of water in a tank with exposure to sub lethal concentration of 8 ppm of cadmium chloride for a

period of 6 days. A control tank too was also set up. Fishes from each tank were sacrificed by decapitation and the kidney tissues were fixed primarily in 3 % glutaraldehyde and then in Osmium tetroxide and processed for electron microscopy. Semi-thin and ultra-thin sections were taken on LKB ultramicrotome and picked up on G-200 copper grids. Semi-thin sections were stained with Toluidine blue for half an hour and ultra-thin sections were stained for one hour with uranyl acetate and counter stained with lead citrate. Semithin sections were seen under the compound microscope and grids were scanned under a Zeiss EM 109 electron microscope. Kidney tissue was also processed for light microscopy where tissues were fixed in Neutral formalin for 24 hours and stained with Haematoxylin eosin [9]. The concentration of Cadmium in tissues was analysed and confirmed using a flame Atomic Absorption spectrophotometer.

3. Results & Observations

The kidney in fish is multi-functional and not only helps eliminate nitrogenous wastes but is also known to possess endocrine, haematopoietic and lymphatic tissue [10]. Ultrastructural features of a uriniferous tubules have been reported to differ in different species [11]. These features vary not only with the species but also with the habits and habitats of animals. Variations are also seen with change in sex of the animal as has been seen in mature sticklebacks, *Gasterosteus aculeatus* [12]. Hence the ultrastructure of fish kidney was studied in *Oreochromis mossambicus* (Peters) to establish the various regions of the uriniferous tubule in fish [13].

Fish kidney when observed under the light microscope presents the structure of a typical vertebrate. In sections, glomeruli with Bowman's capsule, proximal and distal ends of uriniferous tubules and collecting tubules are

distinguishable. Besides the connective tissue cells and blood cells, wandering cells are peculiar cells found in the interstitial regions.



Figure 1: Light microscopic section of TS of Kidney (control) showing different regions of uriniferous tubules. Stain H/E

Key: Ct – collecting tubule; Bc – Bowman's capsule; gl – glomerulus; haem – haemopoietic region; pr – proximal region

In the present study fish was treated with sub-lethal dose of Cadmium chloride (8 ppm for 6 days) and processed for electron microscopy.

Gross changes

Kidney appears pale red and pulpy. Fish shows a shrunken appearance.

Ultrastructural changes

The study has indicated that ultrastructural features of kidney tubules changed drastically with the treatment. Such changes, though vary from region to region, can be generalised as under:

- 1) Presence of some dense material within the lumen. This is especially evident in the distal region of the tubule as well as within the collecting tubule.
- 2) Blocking of junctional complex. This feature is more or less observed in all regions of the tubule. The junctional complexes which are situated at the apical extremities of the adjacent cells appear dark owing to the presence of some dense material within the intercellular space of the region.
- 3) Increase in the number of vesicles both in the apical as well as basal regions. Such vesicles are usually filled with some material.
- 4) Appearance of vacuoles or their increase in number and sizes. This feature is observed in the proximal and distal tubules. However, the condition is seen to a maximum extent within the epithelial cells lining the collecting tubule.
- 5) Fragmentation of endoplasmic reticulum. This condition is seen in almost all regions of the tubule. The fragmented tubules are often seen in dilated condition.
- 6) Appearance of autophagic vesicles and cytosomes within the epithelial cells.

- 7) Plasmalemma invaginations wherever seen are wavy and are often in broken condition.
- 8) Mitochondria are invariably seen in contracted condition. Constrictions often appear at various levels of elongated mitochondria and they show signs of fragmentation. Cristae are also affected. The other change that is observed is the vacuolation of mitochondria.
- 9) Reduction in the number of cytoplasmic granules leading to the pale appearance of the cells.
- 10) Presence of dense amorphous bodies at various levels of the epithelial cells. Such bodies are spherical in outline and are represented in various shapes.

Ultrastructure of kidney in *Oreochromis mossambicus* (Peters) has been described in detail earlier [13]. Ultrastructure of kidney indicates that it consists of 5 distinct regions, namely, neck region, proximal region, central region, distal region and collecting tubule. However, figures of control fish (fig. 2, 4, 5, 7, 10, 15 respectively.) have been shown along with the same part of fish treated with Cadmium Chloride.

Neck region

Apparently no noticeable change is seen in ultrastructure of the cells lining the neck region. Cytoplasmic granules probably are reduced to a certain extent. (Fig 2 control and 3 treated).



Figure 2

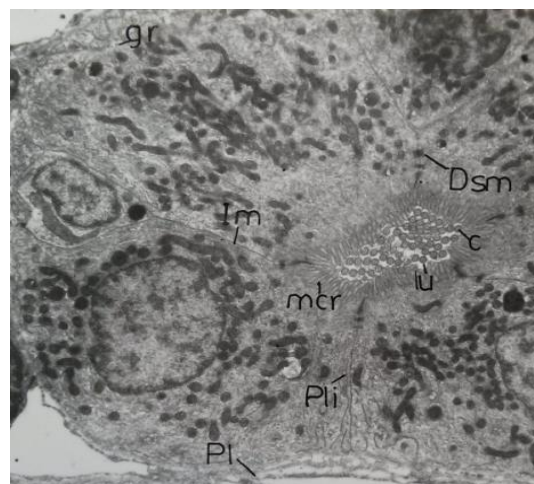


Figure 3

Figure 2 (Control) and 3 (Treated) – Electron micrographs of the neck region of the uriniferous tubule

Key: N – Nucleus; gr – granules; Dsm – desmosomes; c – cilia; lu – lumen; mcr – microvilli; Im – intercellular membrane; Pli – Plasmalemma invagination; Pl – Plasmalemma

Proximal region

Concentration of cytoplasmic granules is reduced. Lumen shows presence of some accumulated material. Apical region of cells shows increased number of vesicles and spherules containing a dense material. Endoplasmic reticulum are dilated. Nuclear membrane is indistinct. There is reduction in number of mitochondria which are shifted more anteriorly. They also show signs of fragmentation (Fig 6).

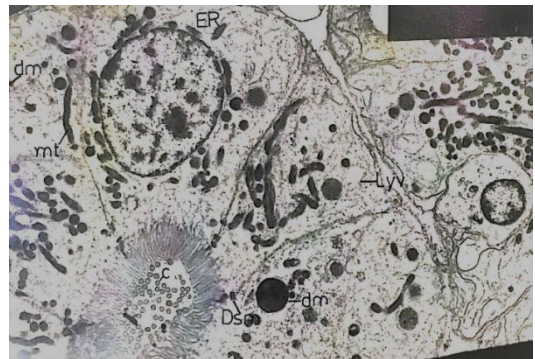


Figure 6: Electron micrograph of the proximal region of the uriniferous tubule (treated 8 ppm, 6 days)

Key: Dsm – desmosomes; mcr – microvilli; mt – mitochondria; ER – endoplasmic reticulum; Lyv – lysosomes vesicles; dm – dense material

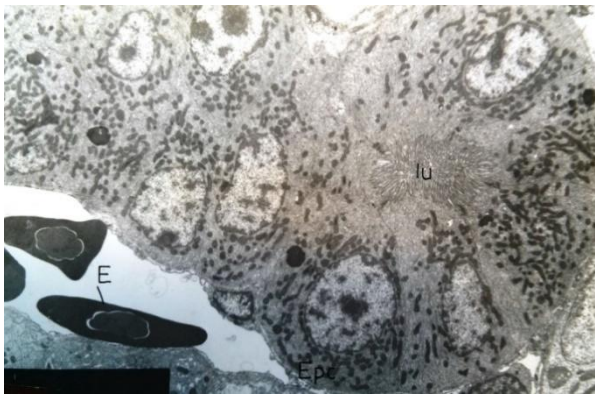


Figure 4

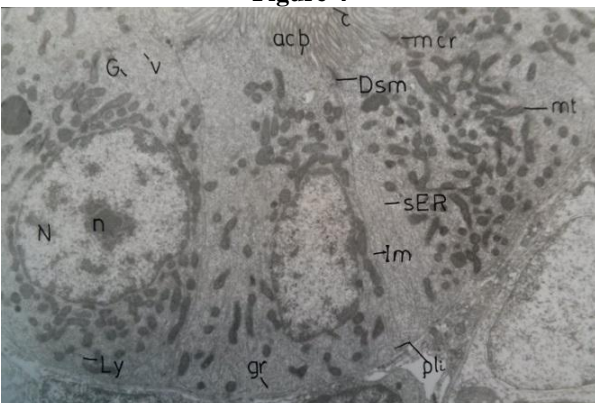


Figure 5

Figure 4 and 5: Electron micrograph of the proximal region of the uriniferous tubule (control) and its magnified version

Key: lu – lumen; Epc – Epithelial cell; E – Erythrocyte; wc – wandering cell; acp – apical ciliated border; c – Cilia; Dsm – desmosomes; mcr – microvilli; mt – mitochondria; gr – granules; v – vesicles; sER – smooth endoplasmic reticulum; Im – intercellular membrane; N – Nucleus; n – nucleolus; Ly – lysosomes; Pli – Plasmalemma invaginations

Central slender region

The apical region of cells shows an increased number of vesicles. Large vacuoles are seen in the central region of nuclei. Nuclei is shifted anteriorly with indistinct nuclear membrane. Mitochondria is highly contracted with presence of vacuoles. Endoplasmic reticulum tubules are often seen fragmented. Plasmalemma invagination is not seen (Fig 8 and 9).

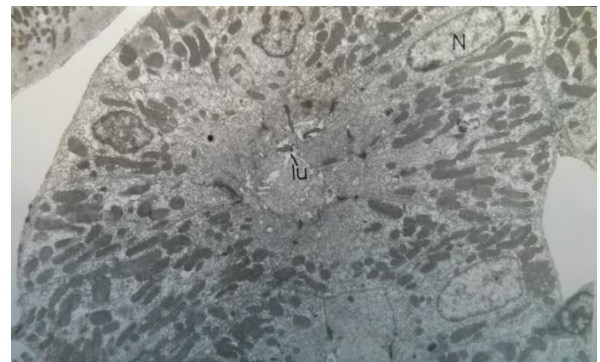


Figure 7: Electron micrographs of TS of slender central region of the uriniferous tubule (Control) and its magnified version

Key: lu – lumen; N – Nucleus

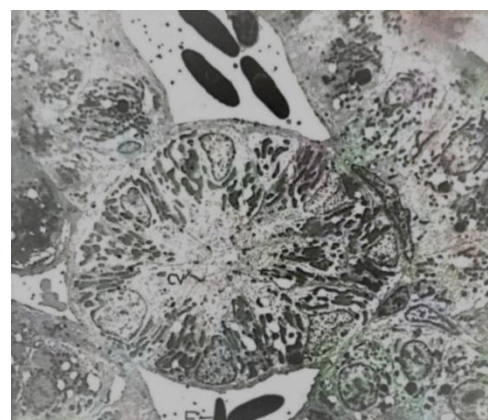


Figure 8

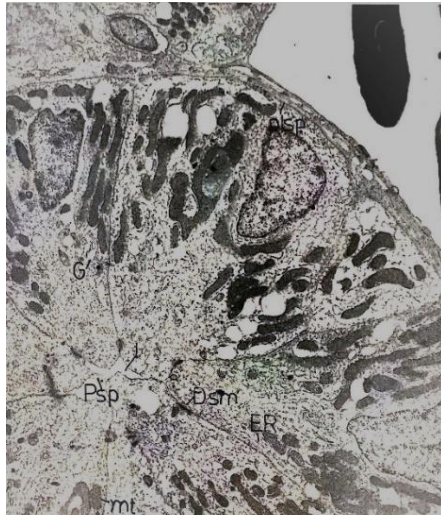


Figure 9

Figure 8 and 9: Electron micrographs of TS of slender central region of the uriniferous tubule (Treated 8 ppm, 6 days) and its magnified version

Key: Cv – cytoplasmic vesicles, E – erythrocyte, ER – endoplasmic reticulum, mt – mitochondria, l – lumen, plsl – plasmalemma, G- Golgi, Psp – pseudopodial process

Distal region

Apical region has a reduced number of granules and few endoplasmic reticulum tubules. Electron dense deposits are seen within the cytoplasm. Mitochondria are shifted anteriorly. The luminal surface of the epithelial cells appear dark owing to the depository material. Cell junctions appear dark due to presence of electron dense material (photo 11, 12, 13 and 14).

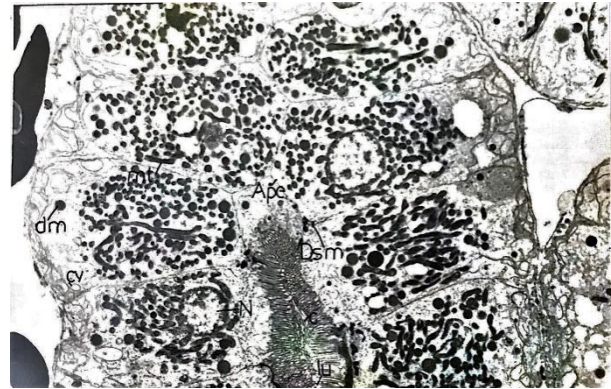


Figure 11

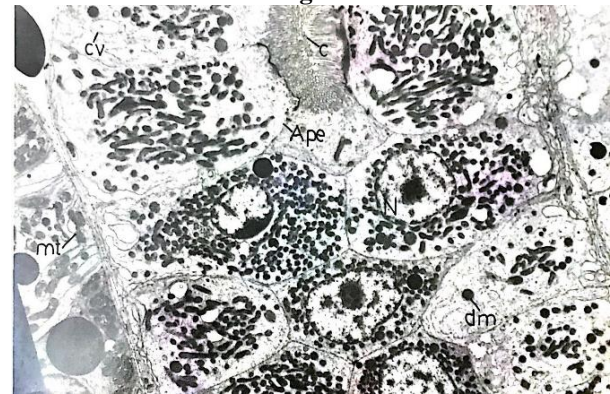


Figure 12

Figure 11 and 12 : Electron micrograph of TS of distal tubule (treated – 8ppm, 6 days)

Key: Cv – cytoplasmic vesicles, Ape – Apical end, c – cilia, mt – mitochondria, dm – dense material, lu – lumen, Dsm – desmosomes

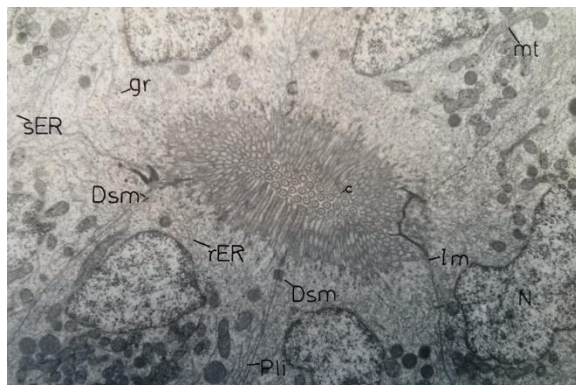


Figure 10 : Electron micrograph of TS of distal tubule (control)

Key: gr – granules; Im – Intercellular membrane; Dsm – desmosomes; N – Nucleus; sER – smooth endoplasmic reticulum; rER – Rough endoplasmic reticulum; Pli – Plasmalemma invaginations; mt – mitochondria

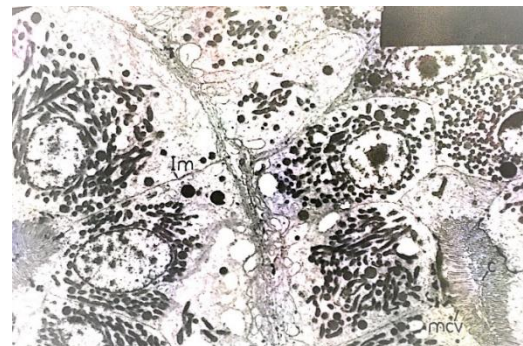


Figure 13

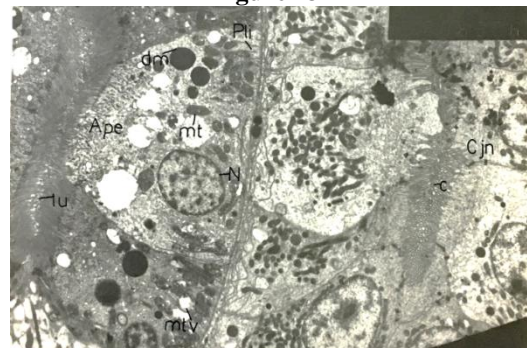


Figure 14

Figure 13 and 14 : Electron micrograph of TS of distal tubule (treated – 8ppm, 6 days)

Key: Ape – Apical end, lu – lumen, Im – intercellular membrane, N – nucleus, mt – mitochondria, mcv –

microvilli, Pli – plasmalemma invagination, c – cilia, cjn – cell junction, dm – dense material, Mtv – mitochondrial vacuoles

Collecting tubule region

Lumen shows presence of some dark material and micro villi are highly constricted. Their tubular nature is indistinct because of presence of dense amorphous material within. Apical region of the cells is full of large vesicles and vacuoles. This region contains a large number of dense spherules. Nuclei are contracted and shifted towards the base. Mitochondria appear pale due to presence of vacuoles. Endoplasmic reticulum is often fragmented. Plasmalemma invaginations are wavy and often in broken condition. Intercellular membranes are indistinct. Cytoplasm has large scale deposition of electron dense material at the basal extremities of the cells. Spherical bodies with dense material is seen. Golgi body is prominent and consists of vesicles (Fig 16 and 17).

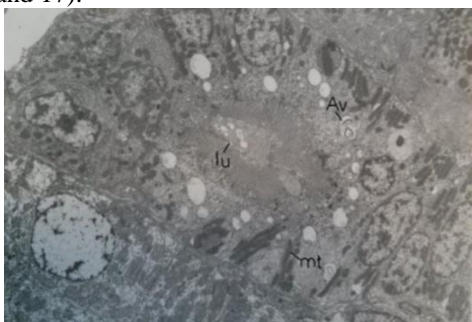


Figure 15: Electron micrograph of TS of collecting tubule (Control) and its magnified version

Key: Av – autophagic vesicle; lu – lumen; mt – mitochondria

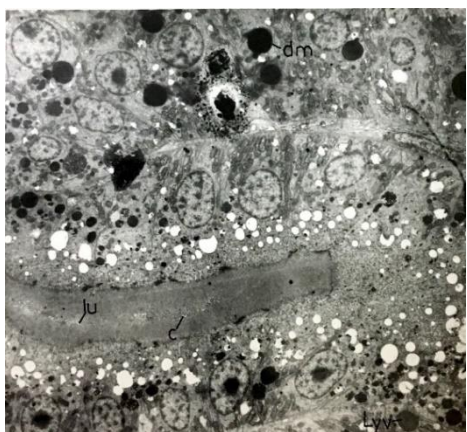


Figure 16

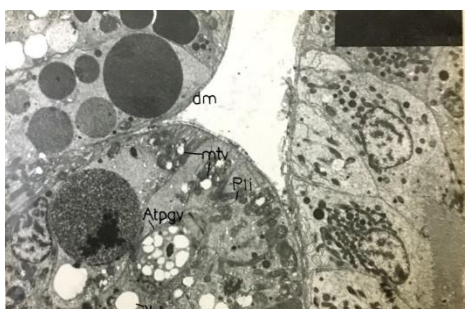


Figure 16 and 17: Electron micrograph of TS of collecting tubule (treated 8 ppm, 6 days) and its magnified version

Key: Atpgv – autophagic vesicle; lu – lumen; mt – mitochondria; dm – dense material; c – cilia; Lyv – lysosomal vesicle; Pli – plasmalemma invagination; v – vesicle

Glomerulii

During the present study few electron micrographs of glomeruli in treated fish only could be obtained. These sections present several of blood cells and together with the cells of glomerular wall. The striking changes observed in such sections are the presence of large number of vesicles with lipid content within the phagocytic cells. Vacuolation of mitochondria of the phagocytic cells is another distinct feature that is observed. Some phagocytic cells have been found to contain large number of dense spherules and autosomes. The other changes if any, could not be studied in the absence of the electron micrographs of normal untreated fish (Fig 18 and 19).

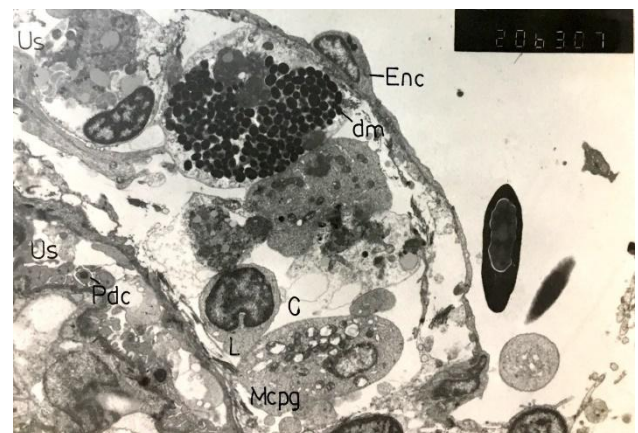


Figure 18

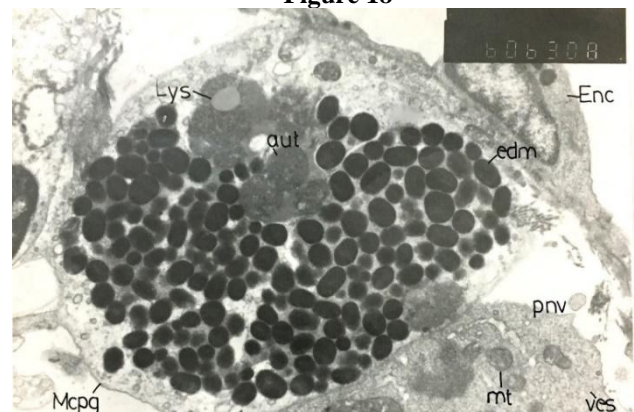


Figure 19

Figure 18 and 19: Electron micrograph of TS of kidney (treated 8 ppm, 6 days)

Key: Us -urinary space; Enc – endothelial cell, C – capillary lumen; L – lymphocyte; Pdc – podocyte; cv – cytoplasmic vacuole; Mcpg – macrophage; dm – dense material; Lys – lysosome; aut – autosome; edm – electron dense matter; Pnv – pinched off vesicle; mt – mitochondria; ves - vesicle

Wandering cells

In electron micrographs of the kidney section of both treated and untreated forms peculiar cells have been seen (besides connective tissue cells and the blood cells) in the intestinal regions. These cells normally occupy the interlobular regions and have been identified as wandering cells [14] [11]. In *Oreochromis mossambicus* (Peters) the cells have

been quite often found to be associated with the proximal tubule. The cells are either spindle shaped or triangular in outline. The cytoplasm is full of dark coloured granules and vesicles. Mitochondria are small, few and spherical. Ribosomes are seen in large numbers. Occasionally a few vesicles are also seen. The cytoplasm has a profile of rough endoplasmic reticulum. Nucleus is massive, triangular or V-shaped and occupies most of the interior of the cell. Chromatin is dense and pycnotic type. In treated fishes, the cells show certain degenerative changes such as vacuolation of the cytoplasm, mitochondria with vacuoles, reduction in the number of cytoplasmic granules accompanied by the appearance of certain irregular shaped vesicles. Similar results were reported by Bulger Ruth Ellen and Trump Benjamin F [11]. Nuclear changes that are observed are indistinctness of the nuclear membrane, nuclear indentation and degeneration of chromatin matter (Fig 20)

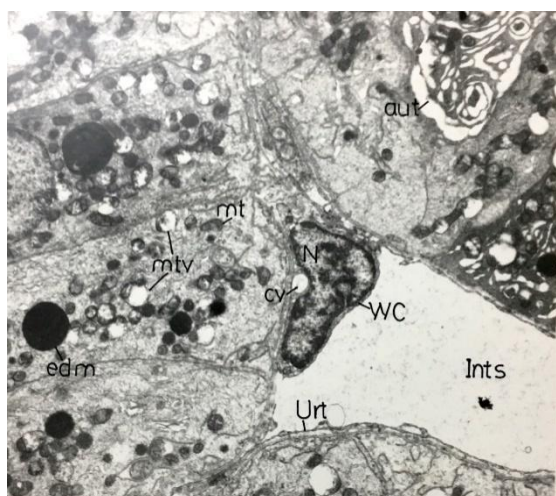


Figure 20: Electron micrograph showing single wandering cell lodged within the intertubular space

Key: aut – autosome; mt – mitochondria; Ints – intertubular space; WC – wandering cell; cv – cytoplasmic vacuole; N – nucleus, mtv – mitochondrial vacuolation; edm – electron dense matter; Urt – uriniferous tubule

4. Discussion

Electron microscopic observation confirms some of the light microscopic observations [15] which are considered to be effects of Cadmium Chloride treatment on uriniferous tubules namely:

- 1) Reduction in no. of cytoplasmic granules from the epithelial cells.
- 2) Appearance of vascular spaces in the region adjacent to basement membrane in particular.
- 3) Presence of occlusion material within the lumen.

The study also confirms the accumulation of cadmium salts within the kidney tubules cells and occasionally within the interspaces between the tubules. The study even confirms the occasional accumulation of pigmented material which has been identified (through histochemical test) to be haemosiderin [17]. In the electron micrographs however both of these appears dense, the former occurs in the form of definite spherules of varying sizes while the latter appears as scattered irregular bodies of dense materials.

The histochemical studies have clearly indicated that the staining properties of different cells gets affected in the sections of treated forms [16] [17] [18]. The electron micrographs of such cells also suggests large scale loss of cytoplasmic granules. This loss of cytoplasmic granules appears to be responsible for the poor staining properties of the cells.

Highly contracted conditions of the nucleus in kidney, degenerative nature of the same and the loss of nuclei are certain other conditions reported in light microscopic observations under similar conditions [19]. Electron Microscopic observations do support the degenerative changes of the nuclei but they do not support the total loss of nuclei. This so-called loss of nuclei in the cells of treated fish therefore may be owing to the failure of the nuclei to get stained.

The other effects of cadmium chloride treatment reported earlier include swelling and the degenerative nature of the endoplasmic reticulum, increased number of vesicles, fragmentation and vacuolation of mitochondria and cytoplasm [20]. Besides, swelling and hypertrophy of tubules with nuclear deterioration and pyknosis was also observed in *Oreochromis niloticus* [18].

Electron microscopic studies confirms some of the treatment changes which includes hypertrophy of bowmans capsule, loss of cellular integrity of renal tubules and their degeneration, few necrotic areas and infiltration of oedematous fluid. Study also reveals the presence of a large no. of macrophages laden with dense spherules, Cytosomes and Cytosegresomes. Most of these changes are observed by others in pollutant induced kidney of fishes. [11] [21] [22] [23]

5. Acknowledgements

Author is grateful to Dr. S.V. Shanbhag for providing valuable guidance for this research; Director, Cancer Research Institute, Parel for providing facilities for electron microscopy.

References

- [1] Ferard J.E., Jouany J.M., Truhant R. and Vasseur P. (1983). Accumulation of Cadmium in freshwater food chain experimental model. *Ecotoxicol. And Environ Safety*. 7(1): 43 – 52
- [2] A.A. Bawuro, B.B. Voegborlo and A.A. Adimado (2018) Bioaccumulation of Heavy metals in some tissues of fish in lake Geriyo, Adamawa State, Nigeria. *Journal of Environmental and Public Health*; Volume 2018, article ID 1854892, 7pgs.
- [3] Ishaq S. Eneji, Rufus ShaAto and P.A. Annune (2011). Bioaccumulation of heavy metals in Fish (*Oreochromis zilli* and *Clarias gariepinus*) organs from river Benue, North-Central Nigeria. *Pakistan Journal of Analytical and Environmental Chemistry*. Vol 12 Issue 1 & 2, pages 25 - 31.
- [4] D. Kumar Babu, J. Obaiah, P. Sudhakar Reddy, G. Bhavani and A. Usha Rani (2009). Combined effect of

- copper, mercury and cadmium induced bioaccumulation in the selected tissues of two aquatic organisms. J. Aqua. Bio; 24 (2), 177-181
- [5] Giari L, Manera M, Simoni E, Dezfuli B (2006). Cellular alterations in different organs of European Sea bass *Dicentrarchus labrax*(L) exposed to Cadmium, *Chemosphere*; 67:1171-1181 [PubMed] Google Scholar
- [6] M.S.Rahman, A.H.Mulla, N. Saha, and A.Rahman (2012) Study on heavy metals levels and its risk assessment in some edible fishes from Bangshi, Savar, Dhaka, Bangladesh. *Food Chemistry*, Vol-134, no4, pp 1847-1854.
- [7] Satrug S, Moore M R (2012). Emerging roles of Cadmium and heme oxygenase in type 2 diabetes and cancer susceptibility. *Tohoku J Exp. Med*; 228; 267-288 [Pub Med] Google Scholar
- [8] American Public Health Association (APHA, 1985) Standard methods for the examination of water and waste water (Washington DC).
- [9] Gurr Edward (1956) A Practical manual of medical and biological staining techniques. Leonard Hill (Books) Ltd. London
- [10] Roberts R.J.(1989). The anatomy and physiology of teleost. In *Fish Pathology*; 2nd edition, pp 13-55 London: Bailliere Tindall.
- [11] Bulger Ruth Ellen & Trump Benjamin F., (1968). Renal morphology of the English Sole *Parophrys vetulus* *American Journal of Anatomy*; 123; pp 195-225
- [12] Hickman Cleveland P.; Jr; and Trump Benjamin F. (1969). The Kidney. *Fish physiology* –Vol I Edited by Hoar W.S. and Randall D.J.
- [13] Meenakshi Sundaresan (Sept. 2014) Ultra Structure of Kidney in the freshwater fish *Tilapia mossambica* (Peters). *European Academic Research*, Vol II , Issue VI, pg. 8563-8579.
- [14] Trump Benjamin F., Bulger Ruth Ellen (1967). Studies of cellular injury in isolated Flounder tubules. Correlation between morphology and function of control tubules and observations of autophagocytosis and mechanical cell damage. *Lab. Invest*, 16 (3): 453-482
- [15] Meenakshi Sundaresan (2019). Cadmium induced toxicity in kidney of freshwater fish, *Oreochromis mossambicus* (Peters). *Proceedings of International conference on Ecology, Ethology & Environment management* ISBN: 978-81-923628-6-1
- [16] Meenakshi Sundaresan (2016). Cadmium induced histological changes in the liver of *Oreochromis mossambicus* (Peters). *European Academic Research* Vol 14 Issue 4, pg 3510-3521
- [17] Meenakshi Sundaresan (2014). Cadmium induced histological and histochemical changes in the spleen of freshwater fish *Tilapia mossambicus* (Peters). *Indian Streams Journal* Vol 4(3)
- [18] Imam A.A. Mekawy, Usama M. Mahmoud, Ekbal T. Wassif and Mervat Naguib (2013), Effects of Cadmium on Some Histopathological and Histochemical Characteristics of the Kidney and Gills Tissues of *Oreochromis niloticus* (Linnaeus, 1758) Dietary Supplemented with Tomato Paste and Vitamin E., *Journal of Fisheries and Aquatic Science*, Volume 8 (5): 553-580.
- [19] Shrivastava A and Pandey A.K (1986) Effect of cadmium chloride on kidney in *Puntius sophore*, a fresh water fish. *Comp. Physiol. Ecol.* Vol 11; no4; pp203-207.
- [20] Authman M. M. N., Zaki M.S, Khallaf E, A and Abbas H.H. (March, 2015), Use of Fish as Bio-Indicator of the Effects of Heavy Metals Pollution, *Journal of Aquaculture Research and Development*, 6:328. doi:10.4172/2155-9546.1000328
- [21] Takashima F and T. Hibiya (1995). An Atlas of Fish Histology: Normal and Pathological features, Second Edition, Kodansha Ltd., Tokyo, ISBN: 9781560814122, pgs 195
- [22] Md. Kawser Ahmed, Elora Parvin, Md. Monirul Islam, Mosammat Salma Akter, Shahneawz Khan & Md. Habibullah Al-Mamun (March 2014) Lead- and cadmium-induced histopathological changes in gill, kidney and liver tissue of freshwater climbing perch *Anabastetus lineatus* (Bloch, 1792) *Journal Chemistry and Ecology*, Volume 30, 2014 - Issue 6
- [23] Saravpreet Kaur, Kuldeep Singh Khera & Jasjit Kaur Kondal (2018), Heavy metal induced Histopathological alteration in Liver, Muscle and Kidney of freshwater Cyprinid, *Labeorohita* (Hamilton). *Journal of Entomology and Zoology Studies*, 6 (2): 2137-2144.