EUROPEAN ACADEMIC RESEARCH
Vol. IV, Issue 4/ July 2016

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Cadmium induced histological changes in the liver of *Oreochromis mossambicus* (Peters)

MEENAKSHI SUNDARESAN[1]
Head and Associate Professor
Department of Zoology
D.G. Ruparel College of Arts, Science and Commerce, Mahim
Mumbai 400 016, Maharashtra, INDIA

Abstract:

Liver of fish represents a pattern typical to that of a vertebrate. In some fishes pancreatic tissue has been found to invade liver tissue along with the portal veins. Liver of CdCl2 treated forms exhibits conditions such as separation and vacuolar degeneration of hepatocytes, condition of necrosis,degeneration of nuclei, hypochromia, appearance of cloudy regions, , increased haemopoietic activity (during early period of treatment), cellular swelling and congestion of blood vessels with the production of occlusion material.

In fishes, pancreatic tissue invades the liver along with blood vessels. Such pancreatic tissue when observed in treated fish indicates signs of tissue separation. Blood cells get destroyed on a large scale with increased concentration of the pollutant and exposure time.

Key words: Vacuolar degeneration of hepatocytes, hypochromia, occlusion material

[1] Dr. **Meenakshi Sundaresan** is Head and Associate Professor in the Department of Zoolgy in D.G. Ruparel College, Mumbai, India. She has been in academics for the past 31 years and her research interests include Cell Biology and Toxicology. She has done extensive research on effect of Cadmium on fish and has 12 publications to her credit.

INTRODUCTION:

Urbanization and development of large scale industries has led to increased water pollution. Anthropogenic contamination of the aquatic environment by heavy metals too has drastically increased in the last several decades. (Kumar *et al.* 2009 & 2007; Ashraj, W. 2005). This eventually has led to bio-accumulation and bio-magnification in the aquatic organisms at all trophic levels, thus becoming a huge cause for concern. Cadmium is one such heavy metal which easily bio accumulates in aquatic organisms like fish leading to histological, pathological and biochemical changes in the gills, kidney, liver and alimentary canal. (Okacha R. C. and Adedeji O. B. 2011, Javed N. and Usmani N. 2011, Puneet Kumar and Anu Singh 2010) Liver and kidney are primary target organs and highest levels of cadmium accumulation were detected in the fish. (Loganathan K. *et al.* 2006, Van Dyk J. C. *et al.* 2005, Thopon et al. 2003 & 2004). Infact Cd causes poisoning in various tissues of animals including man. (Jarup L. 2003).

In the present study, liver of fish has been taken up to study the effects of CdCl2 with respect to histological changes. Fishes were treated to study changes with different dosages of the pollutant for different periods of exposure.

MATERIALS & METHODS:

The experimental fish *Oreochromis mossambicus were* collected from Masunda Lake situated in Thane. They were acclimatized in the laboratory for two weeks. Subsequently the fishes were grouped into five batches of twenty each; one control and others treated with sub-lethal concentration of 1ppm, 2ppm, 4ppm, 6ppm of CdCl2 exposed for 1, 2, 4 and 6 days. They were fed in live Tubifex worms on alternate days. To maintain the concentration of the toxicant the test water was changed every 24 hours. Tanks were aerated with oil free air and test water

quality was evaluated by employing standard methods. (APHA 1985)

Fishes from all five tanks were sacrificed and the liver tissue collected was fixed in neutral Formalin and chilled alcohol. Sections were stained with Haematoxylin/Eosin (Gurr E. 1956) to study the histological changes.

RESULTS AND OBSERVATIONS:

Histological features of fish liver although represents a pattern typical to that of a vertebrate, has a uniqueness atleast in some forms such as carps, sillago and in certain others. In these fish, the pancreatic tissue has been found to invade the liver tissue along with the portal veins. (Hideo A. and Asuka I. 2004, Pai Vinaya L. 1993, Takashi Hibiya 1982) Fig. No. 1 and 2

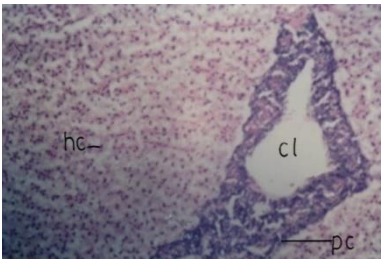


Fig No 1

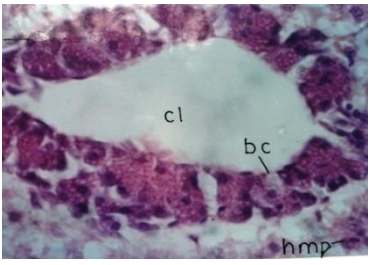


Fig No 2

Section of liver (Control fish): A single blood capillary from the region enlarged to show the pancreatic cells. Stain H/E.

Key

hc – hepatocytes; cl – capillary lumen; bc – blood capillary; hmp – haemopoietic region; pc – pancreatic cell

The liver in fish is mainly composed of a continuous compact field of hepatocytes and scattered with islands of connective tissue enclosing the bile duct and arterial vessels. The hepato-sinusoidal structures of fish liver are cord like in Tilapia.

Gross changes after treatment:

1) Liver of treated fish appears pulpy and pale yellow in colour.

2) It is also shrunken in appearance.

Treatment induced histological changes seen with different dosages are as follows:

1ppm:

Peripheral portion of the liver section indicates presence of vacuoles which gradually increase in number and size as period of exposure increases from day 1 to day 6. Stained pinkish brown but nuclei not seen. The central region appears to be darkly stained from day 4 onwards due to increase in no. of cytoplasmic granules. Cytoplasm surrounding nucleus appears highly irregular with patches of vacuolar degeneration. Hepatic cells located in the interior region are slightly enlarged by end of day 2. Nuclei are stained blue, cytoplasm is lost; hazy and few cracks are seen in the hepatic tissue which gradually increases with exposure period.

Pancreatic tissue cells wherever seen in peripheral region are stained feebly. Gradually cracks appear and cells are with vacuoles. Presence of occlusion material is also found.

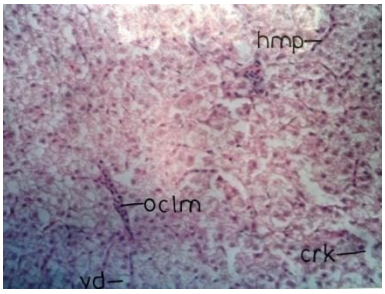


Fig No 3

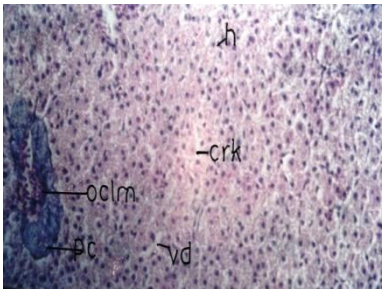


Fig No 4

Section of liver (Treated fish 1ppm 1day & 2 days): Cells appear irregular, nuclei fails to stain, haemopoiesis on the increase. Stain H/E.

Key

vd – vacuolar degeneration; crk – crack; hmp – haemopoietic region; h – hepatocytes; oclm – occlusion material; pc – pancreatic cell

Haemopoietic tissue – Initially appears normal with blood cells giving a clumped look. Gradually increases by day 2 and day 4 and blood vessels start getting constricted. By end of day 6, there is a reduction in haemopoiesis and wherever seen appears as a pink patch. Fig No 3, 4, 5 and 6.

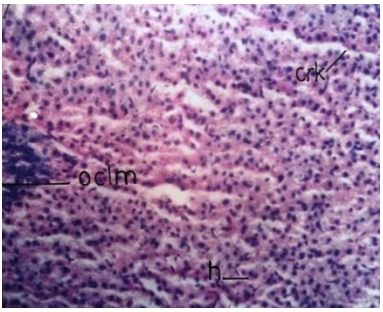


Fig No 5

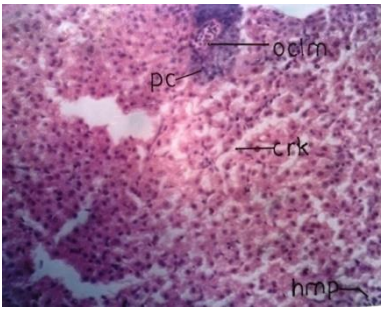


Fig No 6

Section of liver (Treated fish 1ppm 4 days & 6 days): Increase in cracking of tissue evident and capillary shows occlusion material. Stain H/E.

Key

vd – vacuolar degeneration; crk – crack; hmp – haemopoietic region; h – hepatocytes; oclm – occlusion material; pc – pancreatic cell

2ppm:

Nuclear degeneration is seen over peripheral extremities. Stained pale pink. As period of exposure increases, nuclei not visible and the degenerated nuclei appear brown in colour. Rest of the tissue appears cloudy. End of day 4 shows cracks of the tissue with indistinct cell boundaries. The hepatic cells are seen in the form of cords of cells separated from each other. Differential staining of reddish-brown and violet stained areas are seen in the tissue with H/E.

Pancreatic tissue cells stained light and individual cells not clearly seen.

Haemopoietic tissue occurs in the form of eosinophilic patches which initially increase but by end of day 4 and 6, erythrocytes start forming clumps which may be seen in the

lumen of the blood vessels as well as in the haemopoietic tissue. Fig No 7 and 8.

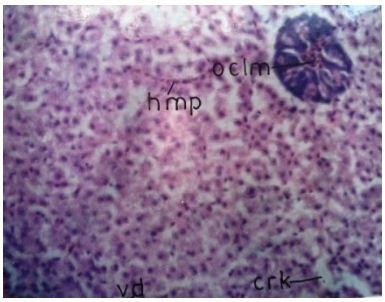


Fig No 7

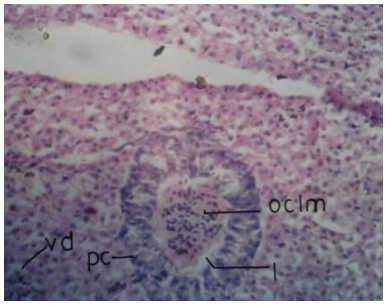


Fig No 8

Section of liver (Treated fish 2ppm 4 days & 6 days): Nuclear degeneration, cracks in tissue, hypochromia and occlusion material. Stain H/E.

Key

Oclm – occlusion material; hmp – haemopoietic region; crk – crack; vd – vacuolar degeneration; pc – pancreatic cell; l – lumen

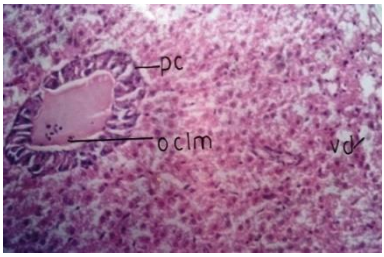
4ppm:

Hepatic cells appear somewhat bulged and spherical. Nucleus with surrounding cytoplasm pushed to one side. Vacuoles start appearing and regions of the liver appear to be in different stages of degeneration. Cloudy regions start appearing. By end of day 2, bulged cells and appearance of vacuoles characteristic more so near the sinuses. By end of day 4, hepatic tissue appears cloudy with indistinct cell boundaries. Cracks start appearing and unequal staining of liver tissue is seen. Pink cytoplasm with blue nuclei or yellowish-brown nuclei is observed with H/E stain. Occlusion material is seen.

Pancreatic tissue cells stained feebly and gradually get separated with cracks appearing by end of day 4 and 6.

Haemopoietic tissue – Blood cells are enlarged, haemopoietic tissue increases, seen with eosinophilic substances, heterogeneous staining property seen. Day 4 shows occlusion material at various sites, especially within the lumen of blood vessels. RBCs are entangled within. On day 6 blood

cells show clumping with eosinophilic material. Fig No 9 and 10.



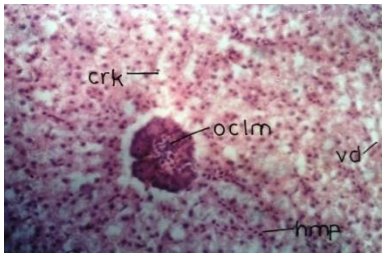


Fig No 9 **Fig No 10**

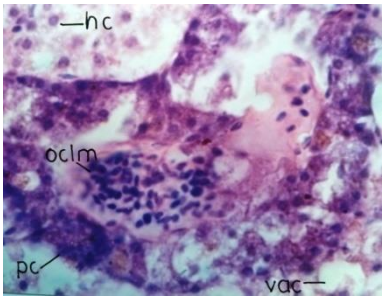
Section of liver (Treated fish 4ppm 2 days & 6 days): Vacuolar degeneration, occlusion material. Stain H/E.

Key

Pc – pancreatic cells; oclm – occlusion material; vd – vacuolar degeneration; crk – crack; hmp – haemopoietic region

6ppm:

Hepatic cells compactly placed, stained feebly, appearance of vacuoles and nuclear degeneration seen in peripheral region. Interior region appears cloudy. From day 2 onwards, degeneration of hepatic cells is seen in the form of cords which are separated from each other. Eosinophilic occlusion seen.



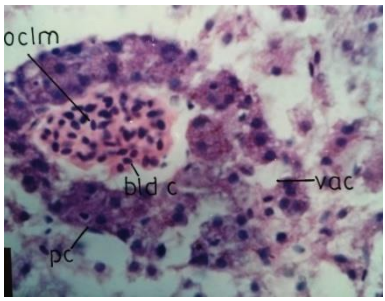


Fig No 11 **Fig No 12**

Section of liver (Treated fish 6ppm 4 days & 6 days): Hepatic cell degeneration, increase in vacuolar spaces, occlusion material present and pancreatic tissue seen with cracks. Stain H/E.

Key

hc – haemopoietic cells; oclm – occlusion material; pc – pancreatic cells; vac- vacuoles; bldc – blood cells;

Day 4 hepatic cells are swollen; cytoplasm shifted to one side giving a stellate appearance. Vacuoles, occlusion material and nuclear degeneration on the increase with cloudy patches. Day 6 similar conditions in aggravated state.

Pancreatic tissue seen with vacuolar spaces on day 1 with cells giving a cloudy appearance. The vacuoles seem to increase with time leading to degeneration of pancreatic tissue. End of day 6 cracks are evident in the tissue.

Haemopoietic tissue is seen at various places. Initially increases followed by occlusion material. By end of day 6 haemosiderin granules and occlusion material very evident. RBCs are destroyed and only theirnuclei are seen. Tissue stains differentially with H/E stain. Fig No 11 and 12.

DISCUSSION:

The study has shown that treatment affects the peripheral region of liver much more in the initial stages, and the effect extends to the central region gradually. The degenerative changes extended to the central region are always dependent on the period of exposure to the pollutant under study. Stained sections of liver of fish that are exposed to shorter periods of exposure when observed show two distinct regions-

A peripheral degenerated region that stains poorly and a central deeply stained intact region. The degenerated region is also characterized by the appearance of cytoplasmic vacuoles. Poor staining property is evident in the nuclear region too. The affected cells have their cytoplasmic vacuoles at the peripheral extremities and as a result, the cytoplasm is restricted to the region around the nucleus and this gives a stellate look to the cytoplasm. With the increased period of exposure, the numbers and sizes of vacuoles are on the increase and this is followed by degenerative changes of the hepatic cell nuclei. In sever conditions; hepatocytes get separated from each other leading to signs of necrosis.

Exposure to pollutant affects the haemopoietic regions of the liver. This is evident from the appearance of eosinophilic granules within the cytoplasm of the erythrocytes. In the forms treated for a longer period, an occlusion material appears at various sites, especially in the lumen of blood vessels. The study has indicated that treatment also affects the pancreatic tissue of the fish. It loses its compactness and shows signs of cell separation. The tissue cells are full of cytoplasmic vacuoles and the number and size of these vacuole are proportional to the dosage of pollutants as well as period of exposure.

In short, most of the ill effects like disruption and separation of hepatic cords, engorgement of blood vessels, congestion, vacuolar degeneration of hepatocytes, hypochromia, necrosis of pancreatic cells, degeneration of nuclei with increased haemopoietic activity during early stages and production of occlusion material have all been reported by earlier workers too (Jalaludeen M.D. et al 2012, Dangre et al 2010, Rangsayatorn N. et al 2004, Rani U.A. and Ramamurthi R. 1989). Heavy metals basically alter the physiological and biochemical parameters in tissues and blood of fish. Most of these pollutants get absorbed and are finally transported to the liver to be stored there or are possibly excreted through gills or kidney. Bio accumulation and bio magnification cause harm not only to fish but the entire ecosystem as these toxic substances knock down the immune, nervous, reproductive and endocrine systems thus affecting them at organ, tissue and cellular level (Geeraerts and Belpaire 2009). Since liver is involved in regulation of many metabolic and physiological processes of the body, it was taken up for this study.

ACKNOWLEDGEMENTS:

The author is grateful to Dr. S. V. Shanbhag for providing valuable guidance for this research; The Principal, Ruia College

for providing laboratory facilities; and to Mr. Biren Daftary for his kind support.

REFERENCES:

-APHA (1985) 'Standard methods for the examination of water and waste water' APHA/AWWA/WPCF, Washington DC, USA.

-Ashraj W. (2005) Accumulation of heavy metals in kidney and heart tissue of Epinephelus micodon fish from tne Arabian gulf. Environmental Monitoring Assessment 1-3(103): 311-316

-**Dangre AJ, Manning S and Brouwer. (2010)** Effects of cadmium and hypoxia-induced expression of hemoglobin and erythropoietin in larval sheepshead minnow, Cyprinodon variegates. Aquatic Toxicol. 99(2): 168-175.

-Geeraerts C. and Belpaire C. (2009) The effects of contaminants in European eel: A review. Ecotoxicology, 19: 239-266.

-**Gurr E. (1956)** A practical manual of medical and biological staining techniques. Leonard Hill (Books)Ltd. London.

-**Hideo Akiyoshi & Asuka Inoue (2004**) Comparative histological study of teleost livers. Zoological Science, 21(8): 841-850. Published by Zoological Society of Japan.

-**Jalaludeen M. D.**, **Arunachalam M.**, **Raja M.**, **Nandagopal S.**, **Showket Ahmad Bhat**, **Sundar S.**, **Palanimutha D. (2012)** Histopathology of the gill, liver and kidney tissues of the fresh water fish *Tilapia mossambica* exposed to Cadmium sulphate: International Journal of Advanced Biological Research: Vol 2(4): 572-578.

-Jarup L. (2003) Hazards of heavy metal contamination, Br. Med. Bull; 68: 167-182.

-Javed M. and Usmani N. (2011) Accumulation of heavy metals in fishes: A human health concern: International journal of Environmental Sciences, 2 (2)

-**Kumar P.**, **Prasad Y.**, **Patra A. K.** and **Swarup D.(2007)** Level of Cadmium and Lead in tissues of Fresh water fish, *Clarias batrachus* and chicken in Western UP, India. Bull. Environ. Contamin. And Toxicol, 79: 396-400.

-**Kumar P.**,**Prasad Y**., **Patra A. K.**,**Ranjan R.**,**Patra R. C.**,**Swarup D.** and **Singh S. P. (2009)**Ascorbic acid, garlic extract and taurine alleviate Cadmium induced oxidative stress in fresh water catfish (*Clarias batrachus)* The Sci. Total Environ. 407: 5024-5030.

-**Loganathan K.**, **Velmurugan B.**, **HongrayHowrelia J.**, **Selvanayagam M.**, **Bhusan, Patnaik B. (2006)** Zinc induced histological changes in brain and liver of *Labeorohita.* (Ham) Journal of Environment Biology: 27: 107-110.

-**Okacha R. C.** and **Adedeji O. B. (2011):** Overview of Cadmium toxicity in fish. Journal of Applied Sciences. Research: 7(7): 1195-1207.

-**Pai Vinaya L. (1993)** On certain histological aspects of fishes, PhD Thesis submitted to university of Bombay.

-**Puneet Kumar** and **Anu Singh (2010)** Cadmium Toxicity in Fish: An Overview – GERF Bulletin of Biosciences 1(1): 41-47.

-**Rangsayatorn N., Kruatrachue M., Pokethitiyook P., Upathan ES, Lanza GR, Singhakaeco S. (2004)** Ultrastructural changes in various organs of the fish Puntius gonionotus fed cadmium enriched cyanobacteria. Environ. Toxicol. 19: 585-593.

-**Rani U. A. and Ramamurthi R. (1989)** Histopathological alteration in the liver of fresh water teleost Tilapia mossambica in response to Cadmium toxicity. Ecotoxicol. Environ. Saf. 17(2); 221-216.

-**Takashi Hibiya(1982)** An atlas of fish histology – normal and pathological features. Kodansha Ltd, Tokyo.

-**Thopon S.**, **Kruatrachve M.**, **Upatham E. S.**, **Pokethitiyook P**., **Sahaphongs, JaritKhvan S. (2003)**: Histopathological altertations of white Sea bass, *Lates*

calcarifer in acute and sub chromic Cadmium exposure. Environ. Poll. 121: 307-20.

-**Thopon S.**, **Pokethitiyook P.**, **Chalermwatk**, **Upatham E. S.**, **Sahaphong S. (2004)** Ultrastructural alterations in the liver and kidney of white Sea bass, *Lates calcarifer* in acute and sub-chronic Cadmium exposure. Environ. Toxicol. 19: 11-19.

-**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 2007; 66: 432-40. Epub 2005 Dec 20.

Cadmium induced histological changes in the liver of *Oreochromis mossambicus* (Peters)