Available online at www.journalijmrr.com

INTERNATIONAL JOURNAL OF MODERN RESEARCH AND REVIEWS

International Journal of Modern Research and Reviews

ISSN: 2347-8314

Volume 1, Issue 1, pp 58-61 December,2013

ORIGINAL ARTICLE

EFFECT OF CADMIUM CHLORIDE ON HISTOLOGICAL CHANGES IN THE LIVER TISSUE OF *Labeo rohita*

***R. Thirumavalavan and J. Ganesan**

Department of Zoology, Annamalai University, Annamalai Nagar-608002, Tamilnadu

Article History:*Received 3rd Dec,2013,Accepted 30th Dec,2013,Published 31st December,2013*

ABSTRACT

The present study is aimed to investigate histology and histophathological alterations cadmium exposed fish Labeo rohita. The experimental fish were exposed to sublethal concentration (1.87 ppm) of cadmium chloride for a period of 14 days at intervals of 7 and 14 days. The glycogen and glucose level were observed in 7 days and 14 days. The hepatocytes are damaged and vacuoles are formed in the liver tissue of labeo rohita exposed to cadmium chloride.The present study is concluded that the exposure of cadmium chloride caused tissue damage in the labeo rohita.

Keywords: Cadmium chloride, Histopathology, *Labeo rohita*

1.INTRODUCTION

Heavy metals are widely found in natural environment mostly representing severe health hazards in organism [Bemennan and Schiesty, 1996]. The toxicological effects of pollution are due to their high persistence and accumulation in the organisms [Goyer, 1996]. Although suitable concentration of heavy metals play a vital role in metabolic pathways when their concentration exceed the threshold level, they act as physiological biochemical and behavioral inhibition in the organisms.

Metals are elements found naturally in aquatic ecosystems due to various processes such as weathering and erosion (Viljoen, 1999). Some of these metals are essential to living organisms in trace amounts (for example copper and zinc). Essential trace elements have a narrow optimal concentration range for growth and reproduction, and both excess and shortage can be detrimental to organisms (Pelgrom *et al.*, 1994), with unusually high concentrations becoming toxic to aquatic organisms (Wepener *et al.*, 2001). Metals are introduced into the environment by a wide range of natural and anthropogenic sources (Wepener *et al.*, 2001) and with anthropogenic being either domestic or industrial (Biney *et al.*, 1994). Heavy metals are often present at elevated concentrations in aquatic ecosystems due to the rapid growth in population (Biney *et al.*, 1994), the increase in industrialization (Biney *et al.*, 1994; Pelgrom *et al.*, 1994),

the increase of urbanization and socio-economic activities, exploration and exploitation of natural resources, extension of irrigation and other modern agricultural practices and the lack of environmental regulations (Biney *et al.*, 1994).

Due to their adverse effects on aquatic ecosystems, it is important to identify the sources and measure the discharges of heavy metals into systems nationwide (Biney *et al.*, 1994). Metals and pesticides, in particular, have an inclination to accumulate and undergo food chain magnification (James *et al.*, 1998). It is thus also important to monitor the bioaccumulation of these metals in a system, in order to assess the possible impact on human health (fish consumed), and organism health (exposure to a pollutant or consumption by predators) (Kotze *et al.*, 1999).

Cadmium is major contaminants of aquatic environments that are toxic towards aquatic organisms (Witeska *et al.*, 1995) even at concentrations found in natural waters (Pelgrom *et al.*, 1994). Metals are present in very low concentrations in natural aquatic ecosystems (Nussey, 1998). The most important heavy metals in water pollution are zinc, copper, lead, cadmium, mercury, nickel and chromium (Viljoen, 1999). Metal uptake by aquatic organisms is a two-phased process, firstly involving rapid adsorption or surface binding, followed by a slower transport into the cell interior.

Corresponding author: ***Dr.R.Thirumavalavan****, Asst. Professor, Dept. Of Zoology, Annamalai University-608002*

Transport of metals into the intracellular section may be aided by either diffusion of the metal ion across the cell membrane or by active transport by a carrier protein (Brezonik *et al.,* 1991; Wepener *et al.,* 2001).

Fishes are relatively sensitive to changes in their surroundings environment. Fish health may thus reflect, and give a good indication of the health status of a specific aquatic ecosystem. Early toxic effects of pollution may, however, only be evident on cellular or tissue level before significant changes can be identified in fish behavior or external appearance.

Fishes are important sources of human nutrition and they Fish represent the higher tropic level in the aquatic food chain. Therefore persistent of toxic chemicals accumulate to a maximum concentration in their body when compared to other organisms in the aquatic environment (Sackmauerova et al., 1977b). Liver is one of the most multifaceted and active organs in higher animals. In a vertebrate body, the liver is the most important target organ as it is the chief metabolic and detoxification center (Bhattacharya and Mukherjee, 1976; Abbasi and Sujata Krishnan, 1993). It is the site for numerous and varied metabolic activities, including synthesis of bile which contains bile salts, bile pigments, cholesterol and lecithin. The kidney as an organ is mainly concerned with the removal of waste materials. Lu, (1985) has reported that most toxicants are excreted through the kidney when exposed to pesticides and heavy metals. The pathological effects of heavy metals on kidney of various animals have been studied by several workers (Rajamanickam, 1992). In fish, as in higher vertebrates the kidney performs an important function related to electrolyte and water balance and the maintenance of a stable internal environment. The kidney excretes nitrogen containing waste products from the metabolism such as ammonia and urea.

2.MATERIALS AND METHODS

Procurement of experimental animal

The fresh water fish *Labeo rohita* were collected from the fish farm located in Puthur, Nagapattinam District, 15 Km away from the Uinversity campus. This fishes were brought to the laboratory and transferred to the rectangular fibre glass tanka (100X175cm) of 500 liters capacity containing chlorine free aerated wellwater.

Acclimatization of animals

The fresh water fish, *Labeo rohita* were limatized for a minimum period of 15 days in the laboratory conditions at room temperature (28±1ºC) before subjecting them for screening test. These fingerlings were fed with artificial food pellets on alternative days and the water renewed every 24 hours. The tanks were rinsed with potassium permanganate or acroflavine (2mg/l) to prevent fungal attack.the fresh water fish, *Labeo rohita* were critically screened for the signs of disease, stress, physical damage and mortality./the injured, severely diseased, abnormal and dead fishes were discarded. The feeding was discontinued 24 hours before the beginning of the experiment to reduce the excretory products in the test trough as suggested by Arrora *et al*., (1972). During the acclimation, the fishes were reared in tank until there was less than 10 percent mortality in 4 days perior to the beginning of the test as suggested by Anderson (1977). The water in the experimental trough was changed daily and also aeration was stopped to avoid the possible oxidation of the toxicants.

Selection of Cadmium Chloride

The toxicant, cadmium chloride was used for the present experimental studies. It is rarely found in pure state, it is present in various types of rocks and soils and in water as well as in coal and petroleum. The sulfate, nitrate and halides are soluble in water. Cadmium (atomic number 48, relative atomic mass 112.40) is a metallic element was used for the present study. It is rarely found in pure state, it is present in various types of rocks and soils and in water as well as in coal and petroleum. Among these natural sources, zinc, lead, copper is the main sources of cadmium. Its mobility in the environment and effects on the ecosystem depend to a great extent on the nature of these salts. Some cadmium salts, such as the sulfide, carbonate and oxides are insoluble in water. The sulfate, nitrate and halides are soluble in water. The speciation of cadmium in the environment is of importance in evaluating the potential hazards. In the present investigation cadmium chloride has been selected for the present experimental study. The physical and chemical properties of cadmium chloride are given below.

Toxicity Studies

To evaluate the acute toxicity studies the static renewal toxicity test were conducted according to the methods recommended by American Public Health Association (1960). In the present investigation the toxicity of cadmium chloride (LC_{50}) for 96 hours were analyzed. The LC_{50} is statistical estimate to the concentration of toxic material in water that kills 50 per cent of the test species, under experimental conditions during a specific time interval. The LC_{50} was used because the concentration required to affect the response in 50 percent of the test animals is more reproducible than any other value (Pickering and Handerson, 1966).The screening test was conducted to avoid delay and to save time and effort. The object of this test is to obtain approximate indication of the concentration of a substance likely to be hazardous to the test fish and fishes in general in their natural environment. The toxicant concentration used in the present series of tests were approximately the wide range of concentration viz., 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 ppm aqueous solutions were prepared. The tests were conducted in the rectangular plastic troughs. The troughs were cleaned well and dried before conducting experiments. Then the tests were conducted by allowing ten fishes of *Labeo rohita* in each plastic trough containing 10 liters of water with particular concentration of the cadmium chloride. The screening tests was continued to assess the concentration at which all fishes survived for 24 hours and likewise the concentration at which most of the fishes died simultaneously (Bansal *et al.,* 1980).Preliminary observation showed that beyond 30 ppm of cadmium chloride all the test fishes died. Therefore the concentration of cadmium chloride falling of within 1 to 30 ppm were prepared and ten number of test fishes were introduced to confined narrow range of concentration viz., 1,2,3,4,5,6,7,8,9,10 ppm of cadmium chloride solutions. The behavioral responses of the fish at various concentration of cadmium chloride were observed at regular intervals to ascertain the impact of the cadmium toxicity on the organism. Individuals in the test medium, which showed no responses to stimulation and those without opercular movement, were removed quickly to avoid cannibalism among the fish. In all tests, mortalities were recorded 96 hours.The LC_{50} values were determined by following the method of Finney (1971). Sublethal studies are helpful to assess the response of the test organisms under augmented stress caused by metals. 96 hr LC_{50} value for cadmium chloride was found at 1.87 ppm. Hence the one tenth of 96 hr LC_{50} value (1.87 ppm) was selected for the present investigation as sublethal concentration.

Experimental design

The toxicant exposure was done by 24 hour or renewal bioassay system. For analysis sublethal toxicity, 3 groups of 10 fish each were exposed separately and cadmium chloride (8.5ppm: 10 % 96 hours LC_{50}). Solution prepared in well water. The experimental medium was prepared by dissolving cadmium chloride at 1.87 ppm having dissolved oxygen 5.8 ppm, pH7.4, water hardness 30.3mg/l (APHA *et al*., 1992) and water temperature 28± 2 C. Each group was exposed to 50 l of the experimental medium. Parallel groups of 10 fish each were kept in separate aquaric containing 50 l of well water as control. Feeding was allowed in the experimental as well as control groups every day for a period of 3 hours before the reneval of the medium throughout the tenature of the experiment.

The experimental fish were exposed to sublethal concentration (1.87 ppm) of cadmium chloride for a period of 14 days at intervals of 7and 14 days. The control and experimental fish were dissected out at the end of each period of exposure and the selected organs such as gill, liver and kidney were dissected out for bioaccumulation. The blood samples were also collected for haematological parameters. The tissues later were processed for histological and histopathological studies.

Validity of Analytical Procedures

Fishes of the same size and weight were used throughout the study. Well water whose characteristics did not change noticeably during the course of study was used. The dissolved oxygen level of the experimental solutions was estimated regularly and an optimum dissolved oxygen level was maintained in all the studies. Test solution was renewed every 24 hours to maintain the same cadmium concentration level during later hours of exposure. The experimental solutions were all prepared just prior to the test. For the experimental analysis the fresh water fish *Labeo rohita* were divided into two groups. One group of fish were maintained as to control medium and another group of fish were exposed to sublethal concentration of cadmium chloride solution,

Histological preparations

For histological studies the test organs were dissected out from the treated and control fish and fixed quickly in Bouin's fluid. After 24 hours of exposure the gill, liver, intestine and kidney were processed by following the standard techniques (Gurr, 1959). The gills were treated with 5 percent nitric acid for 24 hours to soften the cartilage before processing. After dehydration in alcoholic series, the tissues were transferred into absolute alcohol and acetone for completing dehydration and later into xylol for clearing till the material become transparent. The tissues were embedded in paraffin wax (E'merck 58 – 60^0C). Sections were cut at 6 µ thickness and deparaffinised sections were stained in Heidenhains alum-Haematoxylin and counterstained with aqueous eosin for microscopic observations.

3.RESULTS

Histology of control liver

The liver cells are polygonal in outline with some spaces in contact with neighboring cells while others are covered to the blood capillaries, the blood is carried through the liver in the sinusoid (Plate II- 5,6). These are loosely arranged blood channels lined by endothelial cells. The hepatocytes are polygonal or hexagonal in the clearly defined cell membrane. The hepatocytes are intack and the arrangement of the hepatic cells is also unique. The nuclei of the hepatocytes are spherical with a regular surface and show uniform size, shape and orientation. (Fig.1,2)

Histopatholgy of treated liver

The liver of *Labeo rohita* exposed to sub lethal concentration of cadmium chloride shows the drastic changes in the cellular architecture compared to the control fish. The hepatocytes are not uniformed in all regions. The blood vessels are destroyed leaving large spaces with damaged red blood cells. Deterioration of cellular architecture with marked necrosis is also observed. The hepatocytes found usually in groups with conspicuous spaces between them. Hepatocytes possess a markedly thickened nuclear and cytoplasmic membrane which is obliterated at some places.

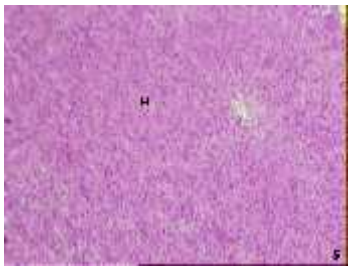

Fig.1 Section of the liver of control fish *Labeo rohita* showing hepatocytes and nucleus:H–Hepatocytes,N- Nucleus

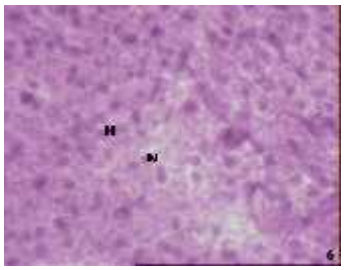

Fig.2.Enlarged portion of control liver showing hepatocytes and nucleus:H–Hepatocytes,N- Nucleus

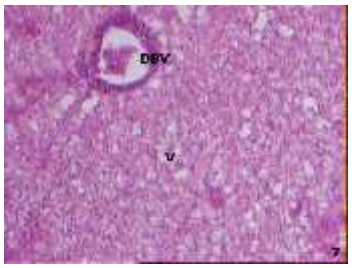

Fig.3.Section of the liver of *Labeo rohita* exposed to cadmium chloride showing damaged blood vessels, scanty cytoplasm, dark hyper- chromatic Nuclei, Pycnotic Nuclei and Vacuoles.DBV – Damaged blood vessels.,DN- Dark hyperchromaticNuclei,V- acuoles

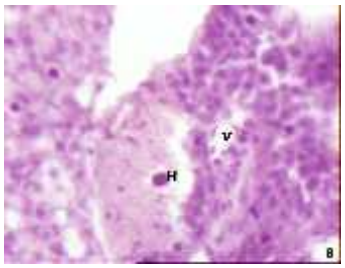

Fig.4.Enlarged portion of liver showing scanty cytoplasm, dark hyper chromatic nuclei, psychotic nuclei and vacuoles;DN- Dark hyperchromatic Nuclei,PN- Pycnotic Nuclei,V-Vacuoles

4.DISCUSSION

The liver has a high concentration of xenobiotic metabolizing enzyme, some of which activate the toxicants to induce leisions locally (Lu, 1985). Toxicants induced changes in the liver tissue of fishes (Coach, 1975). The important abnormality noticed in the liver of *Labeo rohita* after the exposure of sub-lethel concentration of cadmium chloride are the necrosis of hepatocytes, loss of adhesiveness between the cells, enlargement nucleus, vacuolization, karyolysis and swelling of liver cards. The similar results were also made by Bonoard and Hasan (1990). Sukumar (1984) has observed the necrosis, shrinkage of nuclei and cirrhosis of liver of colisalobia when treated with carbofuran. Ramalingam and Reddy, (1981) have reported the shirinkage of nucleus and intra cellular vacuolization in colisc

labia when treated with lindane. Jagadeesan (1994) has also observed the necrosis and shrinkage of nucleus in mercury treated *Labeo rohita.*

The necrosis of hepatocytes, cytoplasmolysis, swelling of liver cord and nuclear enlargement are the important histopathological abnormalities observed in the present investigation. The same results were also reported by Nagarthamma and Ramamoorthy (1982); Jayantha Rao *et al.*, (1983); Gupta and Rajbanshi (1979). Histopathological abnormalities have been observed in the liver of fishes exposed to various metals such as cadmium and zinc in *Anabas testudineus* (Mathivanan, 1988), Lead in *Barbus stigma* (Natarajan, 1979).The histopatholgical abnormalities observed by several workers in different fishes exposed to various metals have deleterious effects which leads to the disfunction of the liver. Since liver is one of the major sites of metabolic activities, the toxic effects of cadmium are more pronounced and cause malfunction resulting in the altered metabolisom of liver in *Labeo rohita*.

5.REFERENCES

Bemennan, R.J. and Schiesty, R.H. 1996. Cadmium is an inducer of oxidative stress in yeast. *Mutat. Res.* 356. 171-178.

Goyer, R.A. 1996. Toxic effects of metals. In : Klassen, C.D (Ed) Casarett and Doull's toxicology: The basic science of poisons. 5th ed. McGraw-Hill, New York.

Viljoen, A. 1999. Effects of zinc and copper on the post ovulatory reproductive potential of the sharptooth catfish *Clarias gariepinus.* M.Sc- thesis, Rand Afrikaans University, South Africa.

Pelgrom, S.M.G.J.; Lamers, L.P.M; Garritsen, J.A.M; Pels, B.M.; and Lock, R.A.C., 1994. Interactions between copper and cadmium during single and combined exposure in juvenile tilapia *Oreochromis mossambicus*: Influence of feeding condition on whole body metal accumulation and the effect of the metals on tissue water and ion content. *Aquat. Toxicol.* 30: 117-135.

Biney, C., Amazu, A.T., Calamari, D., Kaba, N., Mbome, I.L. and Naeve, H. 1994. Review of heavy metals in the African aquatic environment. *Ecotoxico. and Environ. Safety* 31: 134-159.

Wepener, V.; Van Vuren, J.H.J and Du Preez, H.H. 2001. Uptake and distribution of a copper, iron and zinc mixture in gill, liver and plasma of a freshwater teleost, *Tilapia sparmanii*. Water *SA.* 27 (1): 99-108.

Kotze,P ; Du Preez, H.H; and Van Vuren,J.H.J.., 1999. Bioaccumulation of copper and zinc in *Oreochromis mossambicus* and *Clarias gariepinus*, from the Olifants River, Mpumalanga, South Africa. *Water* 25 (1): 99-110.

Rayms – Keller. A.; Olson K.E.; McCraw. M and Beaty, B.J. 1998. Effect of heavy metals *on Aedes aegypti larvae. Exotoxicol. and Environ. safety.* 39: 41 – 47.

James, R.; Sampath, K and Selvamani, P. 1998. Effect of EDTA on reduction of copper toxicity in *Oreochromis mossambicus* (Peters). *Bull. Environ. Contam. Toxicol.* 60: 487-493.

Brezonik, P. L.; King, S.O. and Mach, C.E. 1991. The influence of water chemistry on trace metal bio availability and toxicity to aquatic organisms. In: *Metal ecotoxicology. Concepts and applications*. Eds. Newman, M.C. and McIntosh, A.W. Lewis Publishers, Michigan. 399 pp.

Witeska, M.; Jezierska, B. and Chaber, J. 1995. The influence of cadmium on common carp embryos and larvae. *Aquaculture.* 129: 129-132.

Nussey, G. 1998. Metal Ecotoxicology of the Upper Olifants River at Selected Localities and the Effect of Copper and Zinc on Fish Blood Physiology. *Ph.D-thesis*, Rand Afrikaans University, South Africa.

Viljoen, A. 1999. Effects of zinc and copper on the post ovulatory reproductive potential of the sharptooth catfish *Clarias gariepinus.* M.Sc- thesis, Rand Afrikaans University, South Africa.

Sackmauerova,M. Palusova,O. Szokolay, A and Uhank. J. 1977b. Dynamics of the residues of chlorinated pesticides in the biocenose of the Danube river. *Agrochemia*, 17: 46-48.

Newman, M.C., Jagoe, C.H., 1994. Ligands and the bioavailability of metals in aquatic environments. In: Hamelink, J.L., Landrum, P.F., Bergman, H.L., Benson, W.H. (Eds.), Bioavailability: Physical, Chemical, and Biological Interactions. Lewis Publications, Boca Raton, FL, pp. 39-62.

Mount, D.I 1962, Chronic effects on endrin of bluntnoss minnows and guppies. V.S *Fish wilds Serv. Res. Rep*, 58.

Edward, C.E 1973. Persistent Pesticides in the environment 2nd Ed., Chemical Rubber. co., Cleveland, Ohio , USA.

Fylizer, R. 1978. Toxicity of paraquat to three marine organisms. *Bull. Environ. contam. Toxicol.* 25 (2):283-288

Mahajan, C.L. and singh, J.J. 1973. Water pollution in relation to biology of fishes. Part II. Histopathological charges inducer by synthetic detergents in the gill of *Heteropneustes fossillis*. *Proc. Symp. Environ. Poll.* 18.25.

Arrora, H.C, Sharma, V.P, Chattopathya, S.N and Sinha, L.P. 1972. Bio-assay studies of some commercial organic insecticide part- III, Trials of *Crirhinus mirgala* with 6 insecticides. *Indian J. Env. Hlth*,. 14 (14) : 352 -359.

Abbasi, S.A. and Sujata Krishnan. 1993. The new Japanese pesticide Cartap (Pandan) - Is it as environment friendly as claimed? Global Environmental perceptions series (II) 1 – 151.

Lu, C.Frank, 1985. "Basic Toxicology, " Hemisphere Publishing Corporation, Washington, Cambridge.

Rajamanickam, C,1992. Effects of heavy metal copper on the biochemical constituents, bioaccumulation and histology of the selected organs in the freshwater fish *Mystus vittatus* (Bloch). Ph.D. Thesis, Annamalai University.

APHA, American Public Health Association, 1992. Standard methods for the examination of water and waste water 19th Edition. Washington. DC.

Coach, J.A.1975. Histopathological effects of pesticides and related chemicals on the liver pathology of fishes. Eds. W.E. Ribeln and G.Migaki. The uni. Wisconsin press. Madison.

Bonard and hasan, 1990. Histopathological lesions in the body of catfish *Heteropneustes fossillis* following mercury toxication. *J. Environ Sci. Helth. Port B. Pestic. food contam. Agri. waste*. 25(1): 67- 86.

Sukumar, A. 1984. Effect of carhofuran on histopathology of selects organs in coliga lalia (Ham.) *M.Phil. Thesis.* Annamalai University, India.

Ramalingam, R. and Reddy,S. 1981. Acute Histopathological effects of lindane (T-BHc)on the river of Colisa *Labio Curr. Sci.*, 50(3): 578-580.

Nagarthamma, R. Ramamurthi, R. 1982. Metabolic depression in the freshwater teleost. *Cyprinus carpio* exposed to an organo phosphate pesticide.*curr.sci*. 51(13): 668-669. 3

Jayantha Rao., K. Madhvu.C. Murthy, V.S.R, 1983. Histopathology of malaction of gills of a freshwater teleost, *Tilapia mossambica*. *J.Environ. Biol.*4(1): 9-13.

Gupta, A.K. and Rajbanshi,V.K.1979. Pathological changes resulting from bioassay of copper to *Heteropneustes fossilis* (Bloch.). *Proc. Symp. Environ. Biol.*, 167-172.

Mathivanan, V.1988. Impact of heavy metals cadmium, copper and zinc on a fresh water air breathing fish, *Anabas testudin*eus (Block), Ph.D., Thesis, Annamalai University, India.

Natarajan, A. 1979. Some histopathological and Physiological correlations of lead intoxication in the *Barbas stigma, M.Phil Thesis,* Annamalai University, India.