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ORIGINAL ARTICLE

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

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ABSTRACT

The present study is aimed to observe the histopathologcal changes in the kidney tissue of fresh water fish, Labeo rohita exposed to sublethal concentration of cadmium chloride.The histopathological changes were observed in the kidney tissues of *Labeo rohita* after exposure with cadmium chloride. The present study shows that shrinkage of glomeruli, Bowman's capsule, degeneration of epithelial cells of the renal tubules and vacualization in the cadmium chloride exposed fish

Keywords: Cadmium Chloride, Histology, Kidney, *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; Seymore, 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).

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.

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

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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).

Screening test

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).

Definitive test

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 kidney

Generally, the head kidney is composed of lymphoid, haemotopoietic, interregonal and chromaffin tissues. The chromaffin cells are grouped together in bunches and surrounded by other collageous covering. There is no uniformity in the distribution of these cells, which are found in the peripheral regions. The head kidney is not excretory in function. The trunk kidney is formed of large number of nephrons, each consisting of a renal corpuscle or the Malpighian body and the tubule. The tubule is differentiated into three regions viz., distal, proximal and collecting convoluted tubules.

The inter lobular space is full of lymphoidal tissue which is unevenly distributed. The renal corpuscles are numerous, generally spherical in shape and each contains a highly vascular glomerulus in the normal kidney.

Histopathology of treated kidney

Labeo rohita exposed to sublethal concentration of cadmium produced some conspicuous histopathological changes in the kidney. The distal and collecting convoluted tubules of the kidney undergo degeneration and have a larger lumen due to hypertrophy. The cells of the kidney are destroyed. Vacuolization, shrinkage and breakage of tissue, degeneration of tubular epithelium and swollen nuclei are seen in the kidney. In some areas, the cell boundaries are disrupted and hence, the cells become indistinct. There is an aggregation of blood cells in some areas.

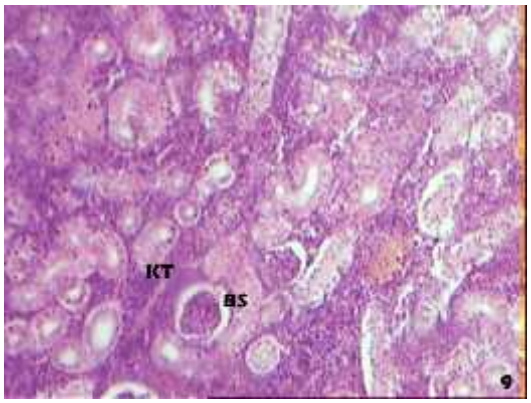

Fig. 1 Section of kidney of control fish *lapeo rohita* showing convoluted tubules, Nucleus CT - Convoluted tubules;N - Nucleus

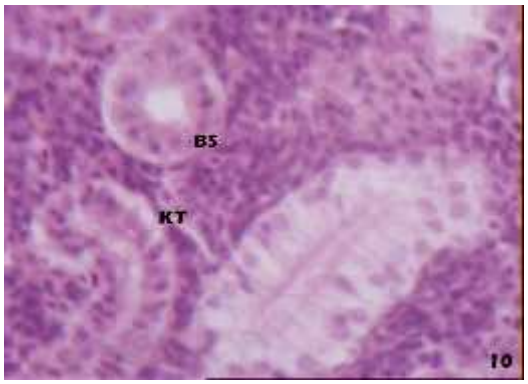

Fig. 2 Enlarged portion of Fig.1 showing convoluted tubules and Nucleus ;CT - Convoluted tubules; - Nucleus

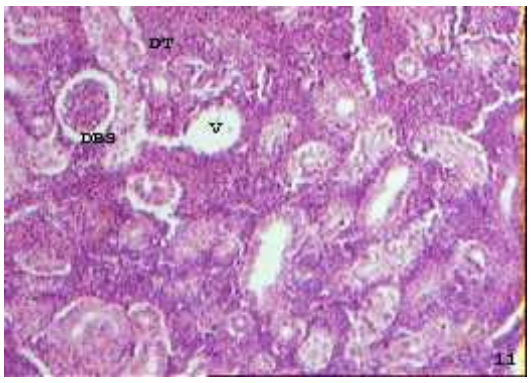

Fig. 3 Section of Kidney exposed to effluent showing damaged kidney tubules, intercellular space, shrinkage of convoluted tubules, pycnotic nucleus add vacuoles.DT- Damaged TubulesPN - Pycnotic nucleus;V - Vacuoles

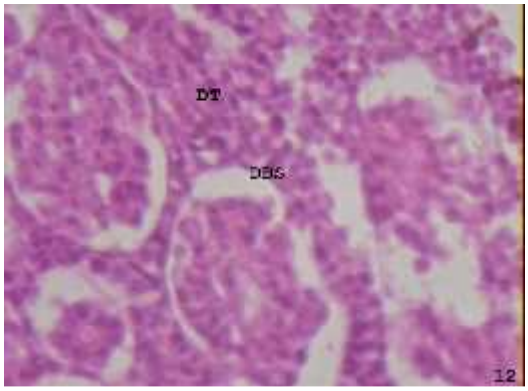

Fig. 3 Section of Kidney exposed to effluent showing damaged kidney tubules, intercellular space, shrinkage of convoluted tubules, pycnotic nucleus add vacuoles.DT - Damaged TubulesPN - Pycnotic nucleus; V - Vacuoles

4.DISCUSSION

The histopathological studies on fish is a note-worthy and promising field to understand the extent to which changes in the structural organization occurs in the organ due to pollutants in the environment. At microscopic level, the cellular organelles lead to alterations in functional systems. Vijayamadhavan and Iwai (1979) have reported that the extent of damage varies with organs, nature of pollutants, medium and test duration. The mortality at fishes occurs due to the pathological lesions caused by mercury. Further critical studies on the histopathological effects at metals on fishes may help to establish the specificity between the metal and their effects.

The kidney is the most sensitive organ with respect to overt toxicity following exposure to heavy metal. The kidney is the main excretory organ, is mainly concerned with removal of waste materials [Lawrence and Mc Cabe, 2002]. Kidney is an extremely complex organ, both anatomicaly and functionally. The primary renal function is the excretion of wastes, but the kidney plays a significant role in the regulation of body homeostasis. Injury to the kidney is induced by chemicals, heavy metals, chromium, anaesthetics, antibiotics, industrial wastes and other miscellaneous nephrotoxins that affect a normal kidney as nephrotoxic. Many reports reveal that kidney is the chief organ where toxic effect is more. Kidney plays a significant role in chemical binding and toxicant concentration capacity than any other organ. This mechanism helps the kidney to remove the toxic materials from the blood. The use of histopathological techniques is a promoting area of research in aquatic toxicology. It gives a real picture of the effects imposed and the involvement of industrial effluent which either disturbs or destroys the vital organs of living organisms.

Labeo rohita exposed to sublethal concentration cadmium shows structural damages in the kidney. The disintegration of kidney tubules, vacuolization, breakage of tissues, aggregation of blood cells in certain areas and necrosis of the kidney tubules have been observed in the present investigation. Similar changes has also been observed by Jagadeesan (1994) in *Labeo rohita* treated with mercuric chloride. Disintegration, vacuolization, breakage of tissue, aggregation of blood cells, necrosis of the kidney tubules have also been noticed in *Mystus vittatus* treated with copper (Rajamanickam, 1992).

The histopathological changes are generally reported in a variety of organisms exposed to different toxicants which support the view that metals cause deleterious effects leading to the disfunctions of kidney as reported in *Anabas testdu bueus* exposed to cadmium, copper and zinc (Mathivanan, 1988), in *Labeo rohita* exposed to mercuric chloride (Jagadeesan, 1994) and in *Punctius conchonius* treated with copper and zinc (Kumar and Pant, 1981). These histopathological symptoms may be due to the hyperactivity of the kidney of *Cyprinus carpio* exposed to the sublethal concentration raw distillery effluent.

Histopathology of kidney has been studied earlier by several researchers on fish when exposed to different pollutants such as sewage treated *Heteropreustes fossilis* (Narain, 1990). In view of the importance of histopathological studies, in the field of toxicology, the effects of raw alcohol distillery effluent on the histology of gill, liver and kidney were studied in the fresh water fish, *Cyprinus carpio* (Linn).Sastry and Sharma (1978) observed that the histopathological effect of arsenic on kidney tissue were enlargement of kidney tubules and damaged tubular epithelial cells in *Channa punctatus treated* with endrin. Similar histological damages in kidney tissue have been reported in *Ictalurus punctatus* exposed to methyl mercury (Kendall, 1975).

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