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HISTOPATHOLOGICAL CHANGES IN THE KIDNEY OF COMMON CARP, *CYPRINUS CARPIO,* FOLLOWING NITRATE EXPOSURE

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Abstract: *Cyprinus carpio,* common carp was exposed to subleathel concentration (12 ppm) of nitrate (KNO_3) under acute and chronic static bioassay conditions. The resultant histopathological changes in the liver were recorded by light microscopy. LC_{50} values of nitrate, according to Reed-Muench method, were 995 ppm for 48 hrs and 865 ppm for 96 hrs. For the acute tests, the fish were exposed to 12 ppm of nitrate for 1,2 and 4 days. For chronic tests, the fish were treated with 12 ppm of nitrate for 8, 16 and 32 days. Control fish were maintained in parallel with the experimental groups. Increase in Bowman's space, degeneration of glomeruli, shrinkage of proximal tubule cells with pycnotic nuclei, increased tubular lumen and increased in intratubular hematopoietic tissue were the most significant changes observed in fish kidney after nitrate exposure. The effects were time dependent being more pronounced in acute treatments. The present investigation illustrates that presence of high concentration of nitrate in water are stressful to fishes.

Keywords: Common carp, histopathology, kidney, $LC_{50,}$ nitrate.

INTRODUCTION

Fresh water is required not only for drinking and other domestic purposes but also for crop irrigation, industrial use and energy production. Sewage water, fertilizers and pesticides used during cultivation, industrial and domestic wastes are making fresh water unsuitable for drinking, recreation, agriculture and industry. The effects of water pollution are not only devastating to people but also to animals, birds and fish [Gottfetied 1993].

Most minerals elements, whether essential or non-essential, found in body have high chemical and biological reactivity. They can be potentially toxic depending upon the dose and other conditions. These elements become contaminants when they are found in foodstuff or in drinking water above nutritionally desirable levels [Bodamer and Murchelano 1990]. Nitrates, nitrites and nitrosamines are chemically and toxicologically related and are therefore generally considered as a group with respect to their toxicological significance [Kardos and Sopper 1974].

The biologically acceptable level of nitrates, according to the WHO standards, in drinking water is 10ppm (10mg l^{-1}). Unfortunately most of the water resources, especially wells, have higher nitrate levels due to contamination of pollutants from one source or the other. Clearly such water resources pose serious health hazards and in some cases are responsible for nitrate poisoning [Burden 1961].

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Accumulation of the chemical pollutants is known to adversely affect the liver, kidney, muscles and other tissues of fish. Kidney a vital organ of body and proper kidney functioning is important to maintain the homeostasis. Kidney is not only involved in removal of wastes from blood but it is also responsible for selective reabsorbtion, which helps in maintaining volume and pH of blood and body fluids, erythropoieses and help in regulating blood pressure by producing the enzyme rennin [Hole 1992]. Kidney is one of those organs, which are severely affected by different toxic chemicals. Necrosis of hematopoietic tissue, vaculoation of tubule cells, dilation of glomerular capillaries and degeneration of epithelial cell lining are some of the pathological changes observed in fish kidney of various toxins by different researchers but there are no studies about the effect of nitrate on kidney histology [Kumar and Pant 1981, Saleh 1982, and Kumari and Banerjee 1986].

In the present study, the pathological effects of nitrate on the kidney of common carp, *Cyprinus carpio*, are described under acute and chronic conditions. This is pioneer study on histopathological effects of nitrate on any organ of fish.

MATERIALS AND METHODS

108 Live specimens of common carp, *Cyprinus carpio,* with an average weight of 36.8g and average length 15.3cm, were collected using cast nets from Punjab Fish Hatchery, Rawal Town Rawalpindi. The fish were transported to the Department of Biological Sciences, Quaid-i-Azam University, Islamabad, and kept in supply tanks. They were allowed to acclimatize to the laboratory conditions for at least one week prior to the start of experiments. The water was constantly aerated with electric aerators. The fish were fed daily on tropical fish food during the acclimation period and were maintained in a photoperiod of 12 hour light and 12 hour darkness using fluorescent lamps and automatic timer clocks placed 24 inches above the water surface. The water temperature was not controlled and it varied with ambient laboratory conditions with an average temperature of 21^{o}C during the experimental period. In order to avoid stress due to crowding, only 6 fishes were placed in each aquarium containing 40 liters of water (total capacity, 60 liters). Food was withheld 24 hours before the start of experiment. The fish thus remained starved during the experimental period.

ESTIMATION OF LC_{50}

As the present study involved acute and chronic laboratory experiments to test the effect of nitrate on *Cyprinus carpio*, the LC_{50} for nitrate was also determined.

Tests for the determination of LC_{50} were based on the mortalities of fish exposed to a series of known concentrations of toxicant, nitrate in this case, with a control in parallel, which received no toxicant. Concentrations

of the toxicant were selected on the basis of preliminary range finding tests for calculation of 48 and 96 hours LC_{50} values according to the method of Reed and Muench [1983]. 72 specimens of *Cyprinus carpio* (of random sex) were exposed to potassium nitrate to determine the LC_{50} of nitrate.

EXPERIMENTAL DESIGN

For acute treatment, fish were exposed to 12 ppm of nitrate for 1, 2 and 4 days and for chronic treatment, fish were exposed to 12 ppm of nitrate for 8, 16 and 32 days. Control (untreated) groups were maintained in parallel. To expose fish to nitrate, nitrate was used as potassium nitrate (KNO_3, Merck). A 1000-ppm stock solution of nitrate was produced by dissolving 1.63g of KNO_3 in 1000ml of distilled water. Diluting the stock solution produced the desired nitrate solution of 8 ppm. This 8-ppm nitrate solution was introduced in 40-liter tap water that already contained 4-ppm nitrate. The aquaria were cleaned and the test concentrations were restored after every 24 hours.

HISTOLOGICAL PROCEDURE

Both treated and untreated fish were stunned by a blow on the head at the end of the specific experimental time duration. The fishes were dissected and slices from the kidney were immersed in fixative sera composed of Glacial acetic acid, Formaldehyde and Ethanol (1:3:7) followed by dehydration in ascending ethanol series and cleared in cedar wood oil. Following infiltration with paraffin, the blocks were sectioned at 6-8 µ thicknesses on a Cambridge microtome. The sections were affixed to precleaned albuminized glass slides and stained with hematoxylin-eosin and photographed under an Optiphot Research Microscope (Nikon).

RESULTS

GENERAL OBSERVATIONS AND BEHAVIORAL RESPONSE

Acute and chronic treatment of fish with 12 ppm nitrate revealed substantial changes in fish behavior. These involved abrupt and sluggish swimming movements in various directions indicating an avoidance response. Occasional jumping and hitting the walls of aquaria were also noted.

As far as general condition of fish is concerned, rapid scale loss, especially from head region, was observed in acute treatment groups. These changes were more pronounced during the initial hours of exposure of fish to nitrate. Surprisingly, defecation by almost all challenged fish was recorded with in 30 minutes of exposure to nitrate Excessive secretion of mucous by treated fish was also observed which was particularly marked in the fish exposed to acute nitrate treatment where aquarium water became cloudy. Thereafter the fish tend to recover

from the disturbed state in due course of time and the frequency of abnormal behavior decreased. However, it stayed higher than in the control group at the end of both acute and chronic exposure.

CONCENTRATION-MORTALITY STUDIES

The LC_{50} value for experimental groups treated with nitrate was 995ppm and 865ppm after 48 and 96 hours treatment respectively (Table 1).

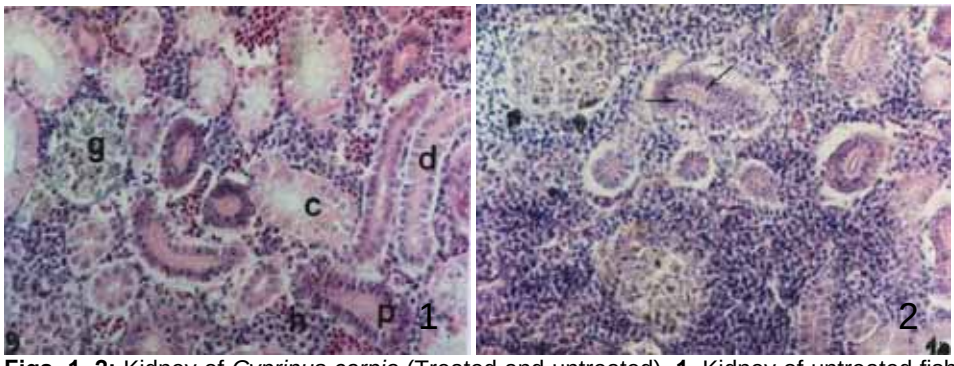
Table 1: LC_{50} and mortality data for *Cyprinus carpio* following treatment with different concentration of nitrate for 48 and 96 hours.

Concentration (ppm)	48-hr (No. of fish died / No. of fish exposed) (% mortality)	96-hr (No. of fish died/No. of fish exposed), (% mortality)
750	0/8 (0%)	2/8 (25%)
800	0/8 (0%)	2/6 (33.3%)
900	2/8 (25%)	6/8 (75%)
1000	2/6 (33.3%)	6/6 (100%)
1100	6/8 (75%)	6/6 (100%)
LC50	995 ppm	865 ppm

HISTOLOGICAL STUDIES

Control

The kidney of *Cyprinus carpio* comprises of functional units, the nephrones. Each of which consists of a renal corpuscle and a renal tubule. The renal corpuscle of nephrone consists of glomerulus and Bowman's capsule. A tubular neck follows the Bowman's capsule. Other regions of the renal tubule are proximal distal and collecting tubules. The interstices of the tubules are enriched with hematopoietic tissue, which contain round to polygonal cells possessing hyperchromatic nuclei (Fig. 1).



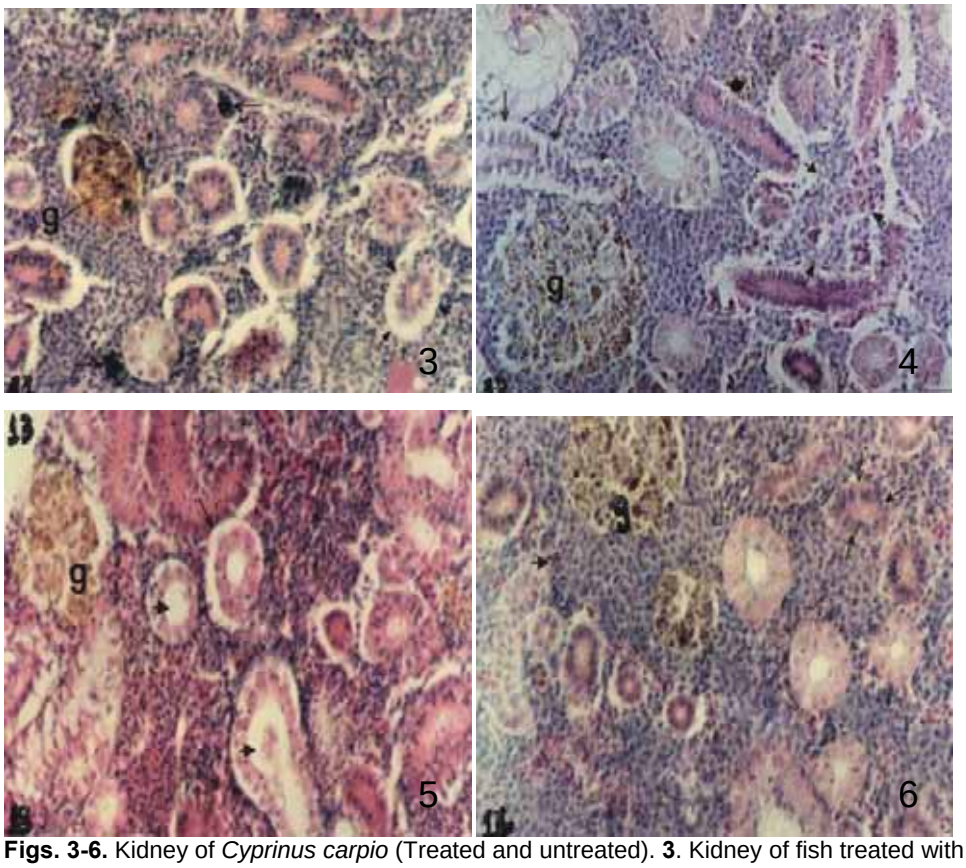
Figs. 1, 2: Kidney of *Cyprinus carpio* (Treated and untreated). **1**. Kidney of untreated fish. C. collecting tubule, d. distal tubule, g. glomerulus, h. hematopoietic tissue, p. proximal tubule. H & E. x 1280. **2**. Kidney of fish treated with 12 ppm of nitrate for 1 day. Note the disintegration of glomeruli. H & E. x 1280.

Acute Exposure to Nitrate

Histopathological analysis followingexposure of fish to 12 ppm nitrate showed no gross morphological changes in the kidney tissue but

progressive degenerative changes in kidney histology were noticed. 1 day exposure of fish to nitrate showed disintegration of parietal layer of glomerulus, disorganization of proximal tubule with pycnotic nuclei and excessive increase in hematopoietic tissue that spread over large area. The nuclei of renal tubules were hyperchromatic and in several cells were displaced to an apical position (Fig. 2).

Exposure of fish to nitrate for 2 and 4 days showed increased disintegration of glomerulus, increase in Bowman's space of glomeruli, vacuolated proximal tubules, enlarged basement membranes of proximal and distal tubule and increased hematopoietic tissue (Figs. 3, 4).



Figs. 3-6. Kidney of *Cyprinus carpio* (Treated and untreated). **3**. Kidney of fish treated with nitrate for 2 days showing enlargement of basement membrane of tubules. H & E. x 1280. **4.** Kidney of fish treated with 12 ppm nitrate for 4 days, vaculoation and distintegration of tubules is evident. H & E. x 1280. **5.** Kidney of fish treated with 12 ppm nitrate for 16 days showing increased tubular lumen. H & E. x 1280. **6.** Kidney of fish treated with 12 ppm nitrate for 32 days showing increased hematopoietic tissue. H & E. x 1280.

Long-Term Exposure to Nitrate

The fish exposed to 12 ppm nitrate for 8, 16 and 32 days survived the entire exposure period and kidney of these fish were morphologically

intact and damage to the kidney was in general less marked than in acute treatments.

The exposure of fish to 12 ppm nitrate for 8 and 16 days resulted in disintegrated glomeruli, disorganized vacuolated and pycnotic proximal and distal tubule cells with largely increased lumen and excessive increasrd hematopoietic tissue that spread over large area (Fig. 5).

After 32 days of exposure, the glomerular tufts became highly melanized and disorganized. Although the tubules did not show disintegration but cellular shrinkage and nuclear pycnosis were noticeable in the proximal na d distal tubules. The hematopoietic tissue showed an increase and occupied wide area between the tubules (Fig. 6).

DISCUSSION

Nitrogen is an element of macromolecules, such as proteins and nucleic acids present in the bodies of organisms. This nitrogen becomes available to animals and humans in the form of nitrates through drinking water or the food chain [Burden 1961]. Above lethal concentrations of nitrates in animal tissues may have lethal effects on the consumers. However, its effects regarding toxicity at histopathological level have not been studied in greater detail. In the present study, the effect of water borne nitrate on kidney of a common carp, C*yprinus carpio*, was studied to assess toxicological significance on the hypothesis that drinking water for humans contain large quantities of nitrates and fish could serve as a very good model system to study its effects on tissues and cells.

The 48 and 96 hour LC_{50} values of nitrate for *Cyprinus carpio* are 995 ppm and 865 ppm respectively. These values differ from LC_{50} values of this metal obtained by various authors. Such differences in toxicity values for different fish species are expected to be due to differential species susceptibilities, differences in experimental conditions and also owing to variable physicochemical properties of water used in the experiments Alkahem 1995].

The sequence of behavioral responses of *Cyprinus carpio*, such as abrupt swimming and sluggish movements on exposure to nitrate agree well with the known effects of various toxicants on different animals [Brungs *et al*. 1973, Kumari and Benerjee 1986]. Sensitivity is one of the fundamental properties of life and all organisms are responsive towards homeostatic disturbance. Abrupt swimming is an example of avoidance response, a reaction to run away from the toxicant. Different scientists also observed similar behavioral changes when they exposed different fish species to various chemicals [Alters 1996, Jafri and shaikh 1999]. The sluggish movements of fish were possible due to toxic action of nitrate on nervous system indicating a stressful, irritating and toxic environment. Secretion of mucous following exposure to nitrate reduces contact with the toxic environment and is liable to reduce skin damage caused by the toxicant [Alkahem 1995].

It is generally considered that nitrate is less toxic to the fish [Burden 1961] but the present histopathological changes observed in the kidney of *Cyprinus carpio* in both acute and chronic conditions of exposure reveals that the severity of effects varies both in intensity and frequency in a time-dependent manner. The effects are far more pronounced in acute conditions than in chronic exposure. It can be stated that in chronic conditions of exposure some sort of repair mechanism takes place in fish kidney [Ahmad and Srivastava 1985]. In general, fish seems to acclimatize, with sub lethal nitrate exposure, in chronic conditions. The present study is thus a pioneer work on the histopathological changes in kidney of *Cyprinus carpio* since no record was available regarding histopathological changes in any organ of a fish due to nitrate exposure. Culturing fish in water containing high nitrate level can cause significant damage to the general health of fish and its culture. As fish was used as a model physiological system during these experiments, so similar results could be expected in human populations due to nitrate exposure, which may lead to glomerulonephritis and similar diseases, which need to be investigated in future.

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