Pakistan J. Zool., vol. 37(4), pp. 297-300, 2005.

Histopathological Changes in the Liver of a Farmed Cyprinid Fish, *Cyprinus carpio,* Following Exposure to Nitrate

FURHAN IQBAL*, IRFAN ZIA QURESHI AND MUHAMMAD ALI

Department of Biological Sciences, Quaid-i-Azam University, Islamabad 45320 (FI, IZQ) and Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan 60800, Pakistan(MA)

Abstract.- LC_{50} of nitrate to *Cyprinus carpio,* according to Reed-Muench method, were 995 ppm for 48-hr and 865 ppm for 96-hr. The fish were treated with 12 ppm of nitrate for 1, 2, 4, 8, 16 and 32 days. Cellular atrophy, pycnotic nuclei,. cytoplasmic vacuolation, widening of sinusoids, disruption of lobular integrity and tissue necrosis were the most conspicuous changes in the liver after exposure to nitrate. The effects were more pronounced In short term treatments.

Key words: LC_{50} of nitrate for fish, Cyprinid fish, *Cyprinus carpio.*

INTRODUCTION

Minerals 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 (Fassett, 1973). The biologically acceptable level of nitrates, according to the WHO standards, in drinking water is 10ppm. Most of the water resources 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).

Accumulation of the chemical pollutants is known to adversely affect the liver, kidney, muscles and other tissues of fish. Liver is an organ that shows primary effects of xenobiotics in general and serves as a good indicator of aquatic pollution (Saleh, 1982).

In the present study, the acute and chronic effects of nitrate on the liver of common carp, *Cyprinus carpio* are described.

* Present address: Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan.

0030-9923/2005/0004-0297 $ 8.00/0

MATERIALS AND METHODS

Live specimens (108) of *Cyprinus carpio,* with an average weight 36.8±5.3 g and length 15.3±3.4 cm, were collected using cast nets from Punjab Fish Hatchery, Rawalpindi. The fishes were transported to the Department of Biological Sciences, Quaid-i-Azam University, Islamabad, and kept in supply tanks. They were allowed to acclimate to the laboratory conditions for at least one week prior to the start of experiments. 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 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±2°C during the experimental period. Only 6 fish were placed in each aquarium containing 40 liters of water (total capacity, 60 liters). Food was withheld 24 hours before the start and during the experiment.

Estimation of LC_{50}

Concentrations of the toxicant were selected on the basis of preliminary range finding tests for calculation of 48-hr LCso according to the method of Reed and Muench (1983).

Experimental design

Fish were exposed to 12 ppm of nitrate for 1, 2 and 4 days for acute treatment and for 8, 16 and

32 days for chronic treatment. Control (untreated) groups were maintained in parallel. A 1000-ppm stock solution of nitrate (KNO_3, E.Merck) was prepared by dissolving 1.63 g of KNO_3 in 1000 ml 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 (N.I.H. Islamabad). The aquaria were cleaned and the test concentrations were restored after every 24 hours.

Histological procedure

The fishes were dissected after given blow on the head and slices of liver were fixed in fixative (Glacial acetic acid, Formaldehyde and Ethanol, 1:3:7) followed by dehydration in ethanol, clearing in cedar wood oil and embedding in paraffin. Then sections (6-8 µm) were cut and stained with hematoxylin-eosin.

RESULTS

General observations and behavioral response

Substantial changes in fish behavior involving abrupt and sluggish swimming movements in various directions with occasional jumping and hitting the walls of aquaria were noted.

Other changes observed include rapid scale loss, especially from head region. These changes were more pronounced during the initial hours of exposure of fish to nitrate. Excessive secretion of mucous by treated fish was also observed which was particularly marked in the fish exposed to acute nitrate treatment. The fish tended to recover from the disturbed state in due course of time and the frequency of abnormal behavior decreased. However, the frequency was still higher than in the control group at the end of both short and long term 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 I).

Table I.- LC_{50} and mortality data for *Cyprinus carpio* following treatment with different concentrations of nitrate for 48 and 96-hr.

Concentration (ppm)	No. of dead fish / No. of fish exposed	
	48-hrs	96-hrs
750	0/8	2/8
800	0/8	2/6
900	2/8	6/8
1000	2/6	6/6
1100	6/8	6/6
LC_{50}	995 ppm	865 ppm

Histological studies

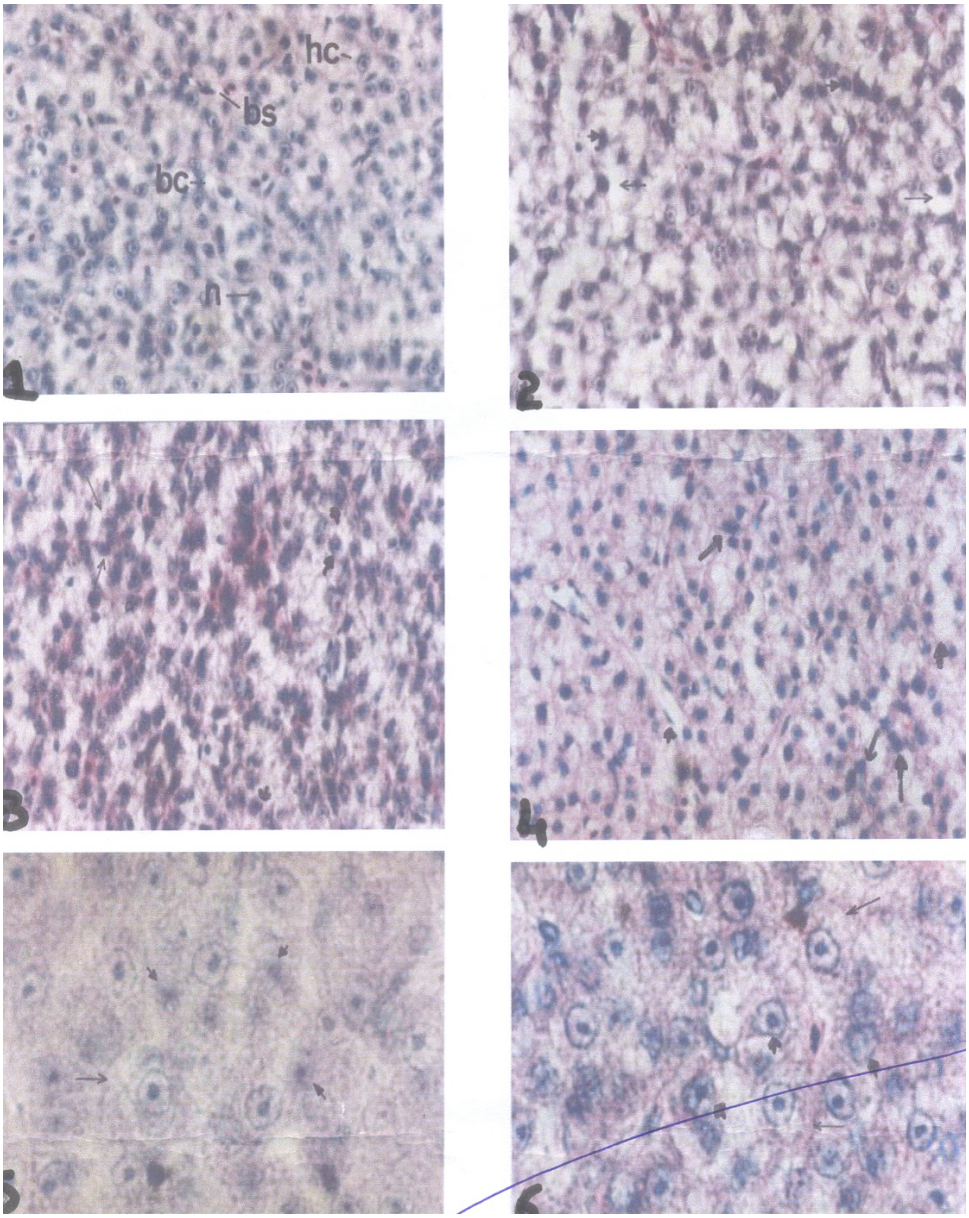
Exposure to nitrate

Exposure of fish to sub lethal concentrations of nitrate, *i.e.* 12 ppm, for 1, 2 and 4 days showed severe degenerative changes in liver with increasing exposure time. The morphology of liver was vague in all cases and compact liver was not observed (as compared to control group Fig. 1) in any of the three treatments as the liver was torn into pieces because of acute nitrate exposure. The widening of bile canaliculi and reduced blood sinusoids were observed in all acute treatments. Exposure of fish for one day resulted in loss of polyhedral architecture of hepatocytes. Lobules were disrupted and hepatocytes showed degeneration. Intensive fatty degeneration was observed resulting in many irregular clear areas between hepatocytes, which were unevenly distributed. Mild hypertrophy and widening of sinusoids were also evident (Fig. 2).

Exposure of fish to nitrate for two and four days resulted in enhanced disruption of cellular architecture, loss of polygonal shape of hepatocytes and appearance of clumps of necrotic tissue. Especially the nuclei of hepatocytes were affected and showed variation in size; some hepatocytes became enlarged while other underwent pycnosis. Fatty degeneration (cytoplasmic vacuolatiion) was persistent but its prevalence decreased as compared to first day treatment (Figs. 3).

Long-term exposure to nitrate

Chronic treatment of fish with12 ppm nitrate for 8, 16 and 32 days provided evidence of improvement of cellular architecture with increasing exposure time. Morphology of liver showed improvement but this effect was less evident as compared to acute treatments. Liver had spongy appearance but it was not torn into pieces.



Figs. 1-6. Histopathological changes in liver of *Cyprinus carpio*: 1, liver of untreated fish showing compact organization. Bc: bile canaliculi, bs: blood sinusoids, hc, hepatocytes; 2-6, liver of fish treated with 12 ppm nitrate for 1 day. Note the disturbed lobular integrity; 3, liver of fish treated with 12 ppm nitrate for 2 days showing clumps of necrotic tissue; 4, liver of fish treated with 12 ppm nitrate for 4 days, pycnotic nuclei and fatty degeneration is evident; 5, liver of fish treated with 12 ppm of nitrate for 16 days showing wrinkled margin of hepatocytes membranes; 6, liver of fish treated with 12 ppm of nitrate for 32 days, atrophy of hepatocytes is evident.
Stain: H&E; Magnification: 1-4 = x1280; 5-6 = x3200.

Most obvious histopathological changes in fish liver exposed to 12-ppm nitrate for 8 and 16 days included necrosis of hepatocytes, mild increase in hepatocyte size, crenated margins of nuclear membrane and fatty degeneration. Minor level of shrinkage of hepatocytes was observed which was

less marked than acute treatments (Fig. 5).

Exposure of fish to nitrate for 32 days resulted in atrophy of hepatocytes, crenated margins of nuclear membranes and fatty degeneration (Fig.6).

DISCUSSION

The 48 and 96 hour LC_{50} values of nitrate for *Cyprinus carpio* are 995 ppm and 865 ppm, respectively. These values are in range of LC_{50} values of nitrate obtained by various authors. 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.,* 1971; Ahmad and Srivastava, 1985; 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. Similar behavioral changes were also observed Alters (1997) and Jafri and Shaikh (1999) when they exposed different fish species to various chemicals. 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 liver and kidney of *Cyprinus carpio* in both short and long term 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 short term conditions than in long-term exposure. The present study is thus a pioneer work on the histopathological changes in liver of *Cyprinus carpio* since no record was available regarding histopathological changes in any organ of a fish due to nitrate. 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 liver cirrhosis and similar diseases.

REFERENCES

AHMAD, G. AND SRIVASTAVA, G.J., 1985. Histopathologic alterations in the liver and skin of a freshwater teleost, *Heteropneustes fossilis* (Bloch) exposed chronically to a subleathel concentration of mehtylene blue. *Pakistan J. Zool*., 17: 239-246.

ALKAHEM, H.F., 1995. Acute and sub1eathel exposure of catfish, *Clarias gariepinus* to cadmium chloride: survival, behavioral and physiological responses. *Pakistan J. Zool*., **27**: 33-37.

ALTERS, S., 1997. *Biology, Understanding life.* Mosby-Year Books, Inc. St. Louis, pp. 836837.

BODAMER, J.E. MURCHELANO, R.A., 1990. Cytological study of vacuolated cells and other aberrant hepatocytes in winter flounder from Boston Harbour. *Cancer Res*., **50**: 6744-6756.

BRUNGS, W.A., LECONARAND, E.W. AND MCKIM, J.M., 1971. Acute and long term . accumulation of copper by brown bullhead, *Ictalurus nebulosus. J. Fish. Res. Bd. Can.,* **30**: 583-586.

BURDEN, E.H.W.J., 1961. The toxicology of nitrates and nitrites with particular reference to the potability of water supplies. *Analysis,* **86**: 429-433.

FASSETT, D.W., 1961. *Nitrates and nitrites*.2nd ed. Committee on Food Protection, National Academy of Science, Washington, D.C.

JAFRI, S.I.H. AND SHAIKH, S.A., 1999. Toxicity and bioaccumu1ation of chromium in Ti1apia *Oreochromis mossambicus* (Cich1idae: Te1eostei). *Pakistan J. Zool.,* **31**: 106-108.

KUMAR, K. AND BANERJEE, V., 1986. Effect of subleathel toxicity of zinc, mercury and cadmium on gills, liver and kidney of *Puntius conchonius* (Hum.). *Indian J. exp. Biol*., **19**: 191-194.

REED, L.J. MUENCH, H., 1983. A simple method of estimating fifty percent end points. *Am. J. Hyg*., **27**: 493-497.

SALEH, H., 1982. Fish liver as indicator of aquatic environmental pollution. *Bull. Inst. Oceanogr. Fish*., **8**: 69-79.

(Received 26 October 2004, revised 7 July 2005)