Pakistan Vet. J., 24(1): 2004

MERCURY INTOXICATION IN GRASS CARP (*CTENOPHARYNGODON IDELLA*)

M. S. Khan, S. A. Khan, Z. I. Chaudhary, M. N. Khan,[1] A. Aslam , K. Ashraf[2], R. M. Ayyub and M. F. Rai.
Department of Pathology, [1]Department of Fisheries and [2]Department of Parasitology, University of Veterinary and Animal Sciences, Lahore, 54000, Pakistan.

ABSTRACT

The present project was carried out to study the effects of acute and chronic mercury intoxication in Grass carp (*Ctenopharyngodon idella*). For acute phase experiment, 48 fish were divided into four equal groups (A, B, C and D). Groups B, C and D were given $HgCl_2$ at sublethal dose as 0.4, 0.5 and 0.6 mg/L, respectively, while group A acted as control. Skin, gills and kidneys were isolated from the fish after 48 and 96 hours for pathological studies. For chronic phase, 72 fish were divided into five groups (E, F, G, H and I), containing 12 fish each except group E which contained 24 fish. Groups F, G, H and I were given $HgCl_2$ at sublethal dose of 0.3 mg/L for 4, 8, 12 and 16 days, respectively, while group E acted as control. Skin, gills and kidneys were isolated from each group (F, G, H and I) after 4, 8, 12 and 16 days respectively for pathological studies. During chronic phase in the treatment groups normal histology of epidermis was disturbed with increased number of immature cells. Overall, skin layers were atrophied and withered. Histopathology of gills showed hyperplasia of epithelial cells of gill filaments, fusion of secondary lamellae giving a club shaped appearance of filaments and contraction and sloughing of respiratory epithelium in groups F, G, H and I. Histopathological examination of kidneys also showed a wide range of toxicity lesions and destruction in treatment groups (F, G, H and I). Disintegration and disorganization of cells of both renal and haemopoitic systems including dilatation of capillaries and thickening of basal lumen were observed. Mild to sever tubular epithelial degeneration, karyolysis, dilation and shrinkage of Bowman's capsule and glomerulus were also observed. In chronic phase experiment, fish showed clinical signs including restlessness, difficult breathing, fin flickering and jerky movements. Suppressed growth rate was also observed in treatment groups (F, G, H and I). During acute phase, after 48 hours, these histopathological lesions were absent in all the groups in all the organs. But after 96 hours, these respective lesions in respective organs were usually of mild, moderate and severe nature in groups B, C and D, respectively. It was concluded that mercury intoxication not only resulted in marked histopathological changes and abnormal clinical signs but also depressed growth rate of fish.

Key words: Fish, Grass carp, mercury intoxication, histopathology, growth rate, clinical signs.

INTRODUCTION

Grass carp (*Ctenopharyngodon idella*) is the herbivorous fish of freshwaters. Because of its good taste and high growth rate, it is cultured in many countries of the world. It was imported in Pakistan from China for the first time in 1964. The purpose of its introduction, in addition to culture, was the biological aquatic weed control in natural waterways, rivers and man-made lakes.

Mercury is released from chlor-alkali, electrical, pharmaceuticals, pesticides, paper and pulp industries (Schaperclaus, 1991). There are two major kinds of mercuric compounds i.e. inorganic and organic. The different industrial effluents release inorganic mercuric compounds like mercuric chloride, which is converted into more toxic organic compound, methyl mercury, through bacterial action (Wiener and Spry, 1996). Then these compounds are transferred to the fish through feed and in this way enter the food chain.

Due to exposure of mercury solutions, epidermis of the fish is damaged and starts secreting profuse quantity of slime (Rajan and Benerjee, 1991). Mercury has also been reported to bind with nucleic acid and inhibit protein synthesis (Mehra and Kanwar 1980). Light microscopic studies revealed severe histopathological changes in kidney, liver and gills due to the impact of pollutants. Histology of kidney showed degeneration of uriniferous tubules indicating impairment of normal functioning of kidney. Bioaccumulation of mercury, copper, cadmium and zinc metals adversely affects liver, muscle, kidney and other tissues of fish. These also disturb the metabolism

of body, and hamper development and growth of the body (Sephar, 1976).

Keeping in view the harmful effects of mercury in fish and ultimately in human life, the effects of acute and chronic mercury intoxication on Grass carp have been studied.

MATERIALS AND METHODS

Experimental fish

For carrying out the experiment, 360 farmed fish *Ctenopharyngodon idella* were brought from a commercial fish farm. Fish were acclimatized for one week before commencement of experiment at pH 7.0 ± 0.1.

Experimental design

Two hundred and forty fish were divided in 12 groups of 20 each with two replicates. LC_{50} of mercuric chloride ($HgCl_2$) was determined with the help of Probit analysis. For this purpose, these 12 groups were given an increasing concentration of $HgCl_2$, i.e. 0.00, 0.10, 0.15, 0.25, 0.35, 0.45, 0.55, 0.65, 0.75, 0.85, 0.95 and 1.05 mg/L, respectively for 96 hours.

Then the experiment was divided into two phases, i.e. acute phase and chronic phase. In acute phase, 48 fish were divided into four groups, A B, C and D, containing 12 fish in each set with two replicates. The groups B, C and D were given different sublethal doses of mercury i.e. 0.4, 0.5 and 0.6 mg/L, respectively, for 96 hours, and group A acted as untreated control.

For the chronic phase, 72 fish were divided into five groups E, F, G, H and I, containing 12 fish each, except group E which contained 24 fish, with two replicates. Fish of groups F, G, H and I were given same sublethal dose of $HgCl_2$ (0.3 mg/L) for 4, 8, 12 and 16 days, respectively. Fish in group E acted as control.

Sampling schedule

For the acute phase of ex-periment sampling was done after 48 and 96 hours. Fish were observed for behavioural and nervous signs, if any. Fish from each group were desensitized by pithing. The skin, gills and kidneys were collected for histopathological examination.

For the chronic phase of experiment, groups F, G, H and I were used on day 4, 8, 12 and 16, respectively, for determination of histopathological changes in skin, gills and kidneys. Fish were weighed on each sampling day to determine growth rate. Fish were observed for behavioral and nervous signs. Parameters under study included fish growth rate, histopathological examination of skin, gills and kidneys, (Drury and Wallington, 1980) and clinical signs. Data were subjected to statistical analysis (unpaired t-test) by following Steel and Torrie (1982)

RESULTS AND DISCUSSION

The results of different parameters noted in different organs regarding both acute and chronic phases are given in Tables 1-7.

Calculation of LC_{50}

In the present study, the 96-hr LC_{50} for mercuric chloride ($HgCl_2$) was 0.8 mg/L. This value is not in line with the findings of Rajan and Benerjee (1991), who reported LC_{50} as 0.3 mg/L in *Heteropneustes fossilis*, which might be due to difference in species. Shakoori *et al*. (1991) reported 96-hr LC_{50} of 0.084 mg/L in small sized *Ctenopharyngodon idella* while Hashmi (1999) observed LC_{50} as 0.15 mg/L. This difference might be due to body size and species variation used in their experiments.

Chronic phase of experiment

Present study revealed decreased growth rate in treated than control fish. Shakoori *et al.* (1991) reported that sublethal doses of mercuric chloride produced severe biochemical abnormalities in blood, liver and muscles that were ultimately reflected through decrease in fish growth. Refusal to feed and appearance of clinically oriented nervous symptoms were the perceptible reasons for the decreased growth rate.

Mercuric chloride intoxication affected both renal and hemopoitic tissues of grass carp. The major histopathological changes in fish included thickening of renal tubules, nuclear pyknosis, degeneration and disorganization of hemopoitic tissue. Many workers support these findings. Banerjee and Bhattacharya (1994) reported degeneration and dispersion of interrenal and chromaffin tissue. This study revealed that $HgCl_2$ affected both endocrine and excretory parts of kidney. Anitha and Sree (1995) also described the effect of $HgCl_2$ on kidney, as intoxication caused degeneration of uriniferous tubules, indicating impairments of normal functioning of kidney. According to Oliveira *et al.* (1996), inorganic mercury caused disorganization of renal tissues.

Mercuric chloride caused histopathological changes in gills. These changes ranged from mild to severe and included hyperplasia of gill epithelium, fusion of secondary lamellae, destruction of respiratory epithelium and formation of club shaped lamellae. Naidu and Ramamurthi (1983) reported buldging of gill lamellae at basal and distal parts. They also reported hypertrophy and hyperplasia of gill lamellae. Prasad (1994a) also reported similar changes in lamellae. These changes included contraction of epithelium, formation of interlamelar bridge and even sloughing of

Table 1: Average fish weight gains in chronic phase

Groups	Average weight gains (g)			
	Day 4	**Day 8**	**Day 12**	**Day 16**
E	1.03*	2.43*	4.48*	6.51*
F	0.31	-	-	-
G	-	0.51	-	-
H	-	-	1.25	-
I	-	-	-	2.18

* = Significant difference ($P<0.05$).

Table 2: Histopathological changes in fish kidney in acute phase

Histopathological changes	Kidney lesion score							
	After 48 hours				After 96 hours			
	A	B	C	D	A	B	C	D
ICS	0	0	0	0	0	1	2	3
NP	0	0	0	0	0	1	2	3
VA	0	0	0	0	0	1	2	3
NA	0	0	0	0	0	0	2	3
ED	0	0	0	0	0	0	1	3

ICS = Intercellular space widening, NP = Nuclear pyknosis in epithelial & haemopoitic tissue,
VA = Vacuolization, NA = Necrotic areas, ED = Epithelial degeneration, EL = Epithelial loosening
Kidney lesion score
0 = Normal, 1 = Mild, 2 = Moderate, 3 = Severe

Table 3: Histopathological changes in fish kidney during chronic phase

Histopathological changes	Kidney lesion score				
	All days	**Day 4**	**Day 8**	**Day 12**	**Day 16**
	E	**F**	**G**	**H**	**I**
DT	0	1	1	2	3
NA	0	0	1	2	3
CT	0	0	1	2	3
ED	0	0	1	2	3
CIF	0	0	1	2	3

DT = Dilatation of renal tubules, NA = Necrotic areas, CT = Connective tissue, ED = Epithelial degeneration, CIF = Cellular infiltration
Kidney lesion score
0 = Normal, 1 = Mild, 2 = Moderate, 3 = Severe

Table 4: Histopathological changes in fish gills during acute phase

Histopathological changes	Gills lesion score							
	After 48 hours				After 96 hours			
	A	**B**	**C**	**D**	**A**	**B**	**C**	**D**
HY	0	1	2	2	0	1	2	3
ED	0	0	1	2	0	1	2	3
DC	0	0	0	2	0	1	2	3
EN	0	0	2	3	0	1	2	3
CL	0	0	0	0	0	1	2	3

HY = Hypertrophy of gills lamellae, ED = Epithelial degeneration, DC = Dilated capillaries,
EN = Epithelial necrosis, CL = Club shaped lamellae
Gills lesion score
0 = Normal, 1 = Mild, 2 = Moderate, 3 = Severe

Table 5: Histopathological changes in fish gills during chronic phase

Histopathological changes	Gills lesion score				
	All days	Day 4	Day 8	Day 12	Day 16
	E	F	G	H	I
HY	0	1	1	2	3
IBE	0	0	1	1	2
CL	0	0	0	2	3
EE	0	0	1	2	3
EN	0	1	1	2	3
DC	0	0	0	0	2

HY = Hypertrophy of gills lamellae, IBE = Interlamelar bridge formation, CL = Club shaped lamellae, EE = Epithelial erosion, EN = Epithelial necrosis, DC = Dilated capillaries

Gills lesion score

0 = Normal, 1 = Mild, 2 = Moderate, 3 = Severe

Table 6: Histopathological changes in fish skin during acute phase

Histopathological changes	Skin lesion score							
	After 48 hours				After 96 hours			
	A	B	C	D	A	B	C	D
ICS	0	0	0	0	0	1	2	3
NP	0	0	0	0	0	1	2	3
VA	0	0	0	0	0	1	2	3
NA	0	0	0	0	0	0	2	3
ED	0	0	0	0	0	0	1	3

ICS = Intercellular space widening, NP = Nuclear pyknosis in epithelial & haemopoitic tissue, VA = Vacuolization, NA = Necrotic areas , ED = Epithelial degeneration , EL = Epithelial loosening

Skin lesion score

0 = Normal, 1 = Mild, 2 = Moderate, 3 = Severe

Table 7: Histopathological changes in fish skin during chronic phase

Histopathological changes	Skin lesion score				
	All days	Day 4	Day 8	Day 12	Day 16
	E	F	G	H	I
AT	0	1	1	2	3
FB	0	0	1	1	2
NA	0	0	0	2	3
ICS	0	0	1	2	3
ED	0	1	1	2	3

AT = Atrophy, FB = Fibrous tissue presence, NA = Necrotic areas , ICS = Intercellular spaces, ED = Epithelial degeneration

Skin lesion score

0 = Normal, 1 = Mild, 2 = Moderate, 3 = Severe

respiratory epithelium at higher doses. Similar histopathological changes in gills are also reported by Oliveira *et al*. (1996), Anitha and Sree (1995) and Hemalatha and Banerjee (1997).

During this investigation, mercury intoxication affected the surface epithelium of fish and caused marked histopathological changes including disintegration and disorganization. Degeneration of all layers of skin and absence of cellular arrangement were noted. Atrophy of all layers of skin was also evident. These findings are in line with the findings of Rajan and Benerjee (1991), who reported degeneration of the cells of skin and disturbance of normal histology of the epidermis. They also reported that the space left behind

by the degenerating cells got quickly filled with haphazardly arranged epithelial cells. Rajan and Benerjee (1992) confirmed the cyclic increase followed by decrease in density, area occupancy and volume of the skin of *Heteropneustes fossilis* due to mercury intoxication. Hypertrophy of the epithelial cells, obstruction of the goblet cells and cellular proliferation were also reported by Oliveira and Torres (1995). Vacuolization, necrosis and pyknosis of the nuclei of the epithelial cells were observed during this investigation. These findings are in line with the findings of Rajan and Banerjee (1993).

Mercury intoxication also caused pronounced clinical symptoms in the grass carp. The nervous symptoms included nervousness, dullness, nudge, yawn and circling. Oliveira *et al*. (1996) reported that the nerves such as the optic, showed disorganized disposition of axons and mainly disruption and dissociation of myelin sheaths, leading to a decrease in motility and increased incoordination. Behavioral disturbances such as off feed and restlessness were also observed. Ribeiro *et al.* (1995) described that olfactory organs were affected in mercury intoxication, which changed the normal behavior of the fish.

Acute phase

During acute phase of this study, after 48 hrs no clinical signs were seen and no histopathological lesions were seen in skin and kidney which are due to very short span of time to affect these organs. But in gills hypertrophy of lamellae, epithelial degeneration and epithelial necrosis were of moderate nature in group C and D. Shakoori *et al*. (1991) reported the same results depending upon the more exposed nature of this organ.

After 96 hrs, in kidney, nuclear pyknosis, necrotic areas, epithelial degeneration and intercellular widening were mild, moderate and severe in groups B, C, and D respectively. In gills hypertrophy of lamellae, epithelial degeneration and epithelial necrosis and formation of club shaped lamellae were of mild, moderate and severe nature in groups B, C and D respectively. Similarly, in skin nuclear pyknosis in epithelium, necrotic ares, ntercellular widening and epithelial degeneration were of mild, moderate and severe nature in groups B, C and D respectively. Shakoori *et al. (1990)* also reported the appearance of these lesions with increasing intensity due to increasing concentration of mercury compounds.

From the above discussion, it can be inferred that mercury intoxication in grass carp (*Ctenopharyngodon idella*) depresses the growth rate, causes marked pathological lesions in organs such as kidney, gills and skin with marked nervous and behavioral symptoms.

REFERENCES

Anitha, K. S. and R. K. N. Sree, 1995. Histo-pathological lesions caused by industrial effluents in kidney, liver and gills of fish *Heteropneustes fossilis* in Hussainsagar Hyderabad, India. Bull. Pure Appl. Sci., 14(2): 57-64.

Banerjee, S. and S. Bhattacharya, 1994. Histopathology of kidney of *Channa punctatus* exposed to chronic nonlethal level of Elsan, mercury, and ammonia. Ecotoxicol. Environ. Saf., 29(3): 265-275.

Drury, R. A. B. and A. Wallington, 1980. "Carleton's Histological Techniques", 5th Ed., Oxford Univ. Press, Great Britain, pp : 40-67.

Hashmi, F., 1999. Effects of mercury intoxication on kidney and hematological changes in *Labeo rohita*. M.Sc. Thesis. Govt. College, Lahore.

Hemalatha, S. and T.K. Banerjee, 1997. Histo-pathological analysis of sublethal toxicity of zinc chloride to the respiratory organs of the airbreathing catfish *Heteropneustes fossilis* (Bloch). Biol. Res., 30(1): 11-21.

Mehra, V. and K. C. Kanwar, 1980. Biochemical changes resulting from intraperitoneal adminis-tration of $HgCl_2$ and methyl mercuric chloride to mice. Toxicol. Lett., 6: 319-326.

Naidu, K. A. and R. Ramamurthi, 1983. Histological observations in gills of the teleost *Sarotherodon mossambicus* with reference to mercury toxicity. Ecotoxicol. Environ. Saf., 7(5): 455-462.

Oliveira, R. C. A. and R. F. Torres, 1995. Acute effects of evaluation of $HgCl_2$ on epidermis of *Trichomycterus brasiliensis.* Ecotoxicol. Environ. Saf., 32(3): 260-266.

Oliveira, R.C.A., E. Fanta, N.M. Turcatti, R.J. Cardoso and C.S. Carvalho, 1996. Lethal effects of inorganic mercury on cells and tissues of Trichomycterus brasiliensis. Biocell., 20(3): 171-178.

Prasad, M.S., 1994a. Effect of short-term exposure to mercuric chloride on the air-breathing catfish, *Heteropneustes fossilis*. I. Light microscopic study of the gill. Biomed. Environ. Sci., 7(4): 327-336.

Prasad, M.S., 1994b. Effect of short-term exposure to mercuric chloride on the air-breathing catfish, *Heteropneustes fossilis*. II. Scanning electron microscopic study of the gill. Biomed. Environ. Sci., 7(4): 337-345.

Rajan, M.T. and T. K. Banerjee, 1991. Histopathological changes induced by acute toxicity of mercuric chloride on the epidermis of freshwater catfish, *Heteropneustes fossilis*.. Ecotoxicol. Environ. Saf., 22(2): 139-152.

Rajan, M.T. and T.K. Banerjee, 1992. Acute toxic effects of mercuric chloride on the mucocytes of the epithelial lining of the accessory respiratory organ and skin of the air breathing catfish *Heteropneustes fossilis* (Bloch). Biomed. Environ. Sci. 5(4): 325-335.

Rajan, M. T. and T. K. Banerjee, 1993. Histopathological changes in the epidermis of the air breathing catfish *Heteropneustes fossilis* exposed to sublethal concentrations of $HgCl_2$. Biomed. Environ. Sci., 6(4): 405-412.

Ribeiro, C.A., L.M. Fernandes, C.S. Carvalho, R.I. Cardoso and N.M. Turcatti, 1995. Acute effects of mercuric chloride on the olfactory epithelium of *Trichomycterus brasiliensis*. Ecotoxicol. Environ. Saf., 31(2): 104-109.

Schaperclaus, W., 1991. Fish Diseases, Vol.1. Oxonian Press New Dehli, India.

Sephar, R. L., 1976. Cadmium and zinc toxicity to flag fish *Jordanella floridae*. J. Fish. Res. Bd. Can., 33: 1939-1945.

Shakoori, A.R., M.J. Iqbal, A.L. Mughal and S.S. Ali, 1991. Drastic biochemical changes following 48 hours of exposure of chinek grass carp to sublethal doses of mercuric chloride. Proc. 1st Symp. Fish and Fishries, Pak., pp: 81-98.

Steel, R. G. D. and J. H. Torrie, 1982. Principles and Procedures of Statistics. A biometrical approach. 2nd Ed., McGraw Hill Book Co. Inc., New York. pp: 137-171.

Talwar, P. K. and A. G. Jhingran, 1991. Inland Fisheries. Oxford and IBH Publishing Co. New Dehli. Vol.1, pp: 175-176.

Wiener, J. G. and D. J. Spry, 1996. Toxicological significance of mercury in Freshwater fish, Lewis Publishers, Baca Raton, Fla. pp : 265-268.

William, S., 1999. Effect of mercury intoxication on liver and hematological changes in *Labeo rohita*. M.Sc. Thesis, Govt. College, Lahore.