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BIOCHEMICAL EFFECTS OF ZINC ON THE MUSCLE OF COMMON CARP, *CYPRINUS CARPIO*

SYED SHAHID ALI, MUHAMMAD AYUB QURESHI, MUHAMMAD JAVED IQBAL AND ABDUL RAUF SHAKOORI

Department of Zoology, University of the Punjab, Quaid-i-Azam Campus, Lahore, Pakistan

Abstract.- Muscles of fresh water fish, *Cyprinus carpio* exposed to zinc chloride (50 µg/ml) for two different time periods (48 hours and 4 weeks) were analyzed for zinc toxicity. The zinc chloride treatment for 48 hours did not produce any significant alteration in muscle enzymes (GOT, GPT and LDH) activities except for CPK which was raised 54 and 59% after 24 and 48 hours of treatment, respectively. Glycogen content exhibit 104 and 54% increase after 24 and 48 hours of treatment, while the total proteins decreased significantly by 21 to 27% at 6, 12 and 48 hours of treatment. In 4 week long zinc chloride treatment almost all the biochemical parameters remained unaffected except for GOT activity which was inhibited 49% after 2 weeks and for FAA content that decreased 59% during 1st week of treatment.

INTRODUCTION

Heavy metals are important component of the industrial waste effluents, released in different freshwater bodies. Unfortunately in Pakistan, which has enough fresh and marine water resources, fresh water animals are the main target of chemical waste coming from the industrial areas. Rivers, canals and lakes are gradually getting polluted by variety of toxic material (Azad *et al.*, 1984; Hakanson, 1984; Ajmal *et al.*, 1985).

Most of the developing countries are interested in developing a suitable and healthy aquaculture system, free from environmental pollutants and their harmful effects. To achieve this target, it is necessary to investigate the hazardous effects of these waste components on aquatic organisms. Several metals (lead, cadmium, mercury) have been analysed in this laboratory for the pathologies they produced on fish blood, liver, muscle and kidney (Shakoori and Ali, 1986, 1987; Iqbal, 1988).

There are at least twenty metals or metal like elements which give rise to well recognised toxic effects in man and its ecological associates including aquatic animals (Duffsu, 1981; Stokinger, 1981; Anadon *et al.*, 1984; Ramamoorthy and Blumhagen, 1984; Correa, 1987; Gonzalez *et al.*, 1987; Monkiewicz *et al.*, 1987; Julshamn *et al.*, 1988). Amongst these heavy metals, zinc is widely distributed in

nature. It is used in various industries such as building hardwares, chemicals, paints, fabrics, plastics, in rubber industry as pigments, in agricultural and commercial machinary, in electrical components, soldering fluxes and batteries, domestic appliances and as protective coating on other metals.

Zinc is an essential micronutrient and is generally regarded as one of the less hazardous elements, though its toxicity may be enhanced by the presence of other contaminants such as arsenic, lead, cadmium and antimony (Duffus, 1981). Variety of toxic effects of zinc have been reported in animals including fish but the literature on metabolic alterations is quite scarce (Speher, 1976; Sastry and Subhadra, 1984; Tort *et al.*, 1984; Beyar *et al.*, 1985; Hilmy *et al.*, 1987; Lu and Combs, 1988). In this study the effects of heavy metal zinc, is being reported on the muscle of *Cyprinus carpio* as this is the major nutritional part of the fish.

MATERIALS AND METHODS

Maintenance of fish

The freshwater fish, *Cyprinus carpio* (common carp, Gulfam) was obtained from the Central Fish Seed Hatchery, Manawan, Lahore and placed in experimental fish pond in big fibre glass tank in the Animal House of the Department of Zoology.

When the fish attained a size of 5-8 cm, it was placed in rounded fibre glass aquaria (2' x 4') in groups where 25±1°C temperature was maintained. Fish was fed on rice polish (rice polish and corn flour, 9:1) @ 50 gm/aquarium every alternate day during the experiments which was prepared according to the formula obtained from Department of Fisheries, Manawan, Lahore. The water in aquaria was constantly aerated by electric aeraters.

Metal administration

Four groups, each of 32 fish in 75 liters of water, were maintained in 4 fibre glass aquaria (2 for control and 2 for metal treatment). After acclimatization of fish for one week, the heavy metal zinc was dissolved in two aquaria, as its soluble salt, zinc chloride @ 50 µg/ml. The water in aquaria was replaced every 72 hours and fresh dose of zinc chloride was administered every time.

The metal was administered to fishes in two experimental aquaria for 48 hours (designated as short term) and 4 weeks (designated as long term experiments). In short term experiment 4 fishes were taken out. 6, 12, 24 and 48 hours after zinc exposure, while in long term experiment, 4 fishes were taken out

every week for 4 weeks. The fishes were quickly knocked down, dissected and their muscle samples stored in freezer -20°C until used later for biochemical analysis.

Biochemical analysis

The weighed amount of muscle was homogenized in 0.89% saline solution in a teflon-glass homogenizer. The homogenate was centrifuged at 3000 rpm for 30 minutes at 5°C and the supernatant was used for the estimation of various activities of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) and concentrations of free amino acids (FAA), soluble and total proteins, glycogen, DNA and RNA contents. The procedures for biochemical estimations have been mentioned in Shakoori *et al.* (1988), except glycogen which was extracted and estimated according to the method of Shibko *et al.* (1967).

RESULTS

Tables I and II show the effect of zinc chloride administered for 48 hours and 4 weeks, respectively on the muscles of fish. Zinc chloride treatment at this dose level has produced moderate changes in the fish muscle.

TABLE I.- PERCENT CHANGE (+, INCREASE, -: DECREASE) IN SOME BIOCHEMICAL COMPONENTS OF FISH MUSCLE EXPOSED TO ZINC CHLORIDE (50 µg/ml) FOR 48 HOURS.

Parameters[a]	Hours of treatment			
	6	12	24	48
CPK	-5.41	-0.25	+53.55*	+59.19*
GOT	-7.48	-25.64	+19.41	+21.90
GPT	+4.69	-8.67	+23.75	+9.25
LDH	-7.40	-0.82	+3.32	-21.89
FAA	-18.19	-15.00	-48.72*	-44.11*
Glycogen	-37.26	+32.25	+104.30*	+53.63*
Soluble protein	-3.45	-13.44*	+42.97	-10.96
Total protein	-20.64*	-21.17*	-12.70	-27.44*
DNA	+20.73	+10.42	+40.51	+31.52
RNA	+27.23	-7.06	-19.56	-19.51

[a]CPK, creatine phosphokinase; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; LDH, lactate dehydrogenase; FAA, free amino acids.
*Student's 't' test, $P<0.05$.

Uninterrupted zinc chloride treatment for 48 hours and 4 weeks did not produce any significant change in both the transaminases (GOT and GPT). The activities when compared with their respective control, ranged between 1.05±0.31 to 1.47±2.24 for GOT and 0.54±0.03 to 0.89±0.23 IU/g for GPT (n=32) except 49% decrease at 2 week which normalized gradually on the 3rd and 4th week. Similarly no variation in muscle LDH activity was found in both short and long term experiments. The CPK activity, on the other hand was elevated by 54 and 59% at 24 and 48 hours of zinc chloride administration, respectively and attained normal level in long term treatment (Table II).

TABLE II.- PERCENT CHANGE (+. INCREASE, -: DECREASE) IN SOME BIOCHEMICAL COMPONENTS OF FISH MUSCLE, EXPOSED TO ZINC CHLORIDE (50 µg/ml) FOR 4 WEEKS.

Parameters[a]	Weeks of treatment			
	1	2	3	4
CPK	-20.00	+3.05	-19.71	-22.18
GOT	-18.11	-48.57*	+11.51	+6.01
GPT	+4.59	-7.61	-24.49	+29.06
LDH	-6.52	-4.29	+15.92	+28.68
FAA	-58.62**	-28.57	-21.43	+3.70
Glycogen	+9.30	+3.92	+48.35	-32.82
Soluble protein	-15.36	-23.25	+17.95	+10.75
Total protein	-14.50	-2.99	-4.00	-12.65
DNA	+23.81	-9.78	-18.72	+21.35
RNA	-13.41	-3.65	-14.62	-18.60

Student's 't' test, *P<0.05; **P<0.01.

In addition to these enzyme activities, some other biochemical components also showed slight deviations. An important storage carbohydrate, glycogen showed 104 and 54% increase over the normal range (12.54±4.32 to 19.50±3.58 mg/g of liver) after 24 and 48 hours of treatment. When metal treatment was extended upto 4 weeks no significant change was noticed. The fish muscle showed 77.75±3.54 to 98.59±4.80 mg/g of total protein in different control groups which decreased from 21-27% during 48 hours (Table I) and remained constant in the remaining experimental period (Table II). Similar alteration was also observed in

FAA content of fish muscle which indicated 48 and 44% decline after 24 and 48 hours and 59% decline at 1st week of uninterrupted zinc chloride treatment when compared with their respective normal range (0.27±0.08 to 0.40±0.11 mg/g). The changes in muscle soluble protein, DNA and RNA contents remained statistically non significant (Tables I and II).

DISCUSSION

The muscle of fish, *Cyprinus carpio* did not indicate any severe alteration in different enzymatic activities in both short term and long term treatments.

The activities of both transaminases (GOT and GPT) and LDH remained unaltered when fish was exposed to zinc chloride for a total period of 48 hours and 4 weeks, except for 49% decrease after two weeks treatment. These results indicate that zinc at this dose level is almost non-toxic so far as muscle chemistry is concerned. The interconversion of lactate into pyruvate and vice versa and gluconeogenesis in the muscle remained unchanged after zinc treatment. Shakoori and Ali (1987) reported increase in muscle LDH activity within 144 hours of cadmium chloride exposure (50 μg/ml) to fish, *Cirrhina mrigala*. Muscle LDH activity remained unchanged with another heavy metal (lead acetate) in a similar study (Shakoori and Ali, 1988). The CPK is another important enzyme in the muscle, the activity of which is increased after muscular damage. Zinc chloride treatment for 48 hours produced a significant rise in CPK activity at the later half of the experiment. The activity increased 53% and 116% after 24 and 48 hours of zinc chloride treatment, respectively, which was recovered when the exposure was extended to 4 weeks. This rise in CPK activity is an indication of increased energy demands of fish which may be required for the induction of defence system of the body to counter the harmful effects of this metal on muscle. Wilhelm *et al.* (1984) and Correa (1987) reported structural and physiological changes in muscle of fish by zinc exposure. Another study from this laboratory which deals with the effect of cadmium chloride on the muscle of fish, *Cirrhina mrigala* showed decreased CPK activity at 50 μg and 100 μg/ml concentrations (Shakoori and Ali, 1987).

Amongst the biochemical components, in addition to enzymes, the muscle glycogen content increased after zinc chloride treatment. This increase (104% and 53%) was found only in short term experiment. It was perhaps either due to the increased glycogen synthesis (glycogenesis) or decreased breakdown or its utilization (glycogenolysis). Hilmy *et al.* (1987) showed increase in muscle glycogen content after zinc treatment, while Bengeri and Patil (1987) showed reduced glycogen content at 65 ug zinc/ml of water in *Labeo rohita*. Muscle glycogen content showed decreasing trend with heavy metal (cadmium chloride)

after short and long term treatments (Shakoori and Ali, 1987). The muscle soluble protein content showed 43% rise within 24 hours of zinc chloride exposure, while the total proteins decreased 48 hours after short term zinc exposure. The FAA contents did not change at all in both short term and long term treatments. As far as the effect on protein are concerned, variable results were reported with other heavy metals (such as cadmium and lead) exposure to fish, *Cirrhina mrigala* from this laboratory. The FAA and soluble protein contents increased with cadmium chloride, while decreased with lead acetate exposure (Shakoori and Ali, 1986, 1987). The muscle DNA and RNA contents remained totally unchanged after exposure of fish to zinc chloride for a total period of 48 hours and 4 weeks which proved that zinc, in these experimental condition, is harmless to the genetic material in this tissue. It is clear from the findings that increase in total proteins in 48 hour experiment is not due to RNA which remained uneffected during this experiment.

The laboratory investigations presented here indicate that zinc chloride under these conditions of dose and duration is relatively less toxic to this fish, *Cyprinus carpio*. However, its toxicity may be increased at higher doses or in combination with other heavy metals, the condition which is more related to our environment where in industrial waste effluent these heavy metals are found in combination with other components. In these conditions the metals can interreact with each other and maysnergise or antagonize the toxic effects on fish (Anadon *et al.*, 1984; Ramamoorthy and Blumhagen, 1984; Monkiewicz *et al.*, 1987; Patimah, 1988).

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