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ZINC-INDUCED BIOCHEMICAL ALTERATIONS IN THE LIVER OF COMMON CARP, *CYPRINUS CARPIO*

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Abstract: Fresh water fish, *Cyprinus carpio* was exposed to zinc chloride (50 g/ml) for 48 hours (short term experiment) and 4 weeks (long term experiment). The liver samples were taken out at 6, 12, 24, and 48 hours in first case and at 1,2,3, and 4 weeks duration in second case, and analysed for biochemical changes. In short-term experiment hepatic AP, AcP and GOT activities decreased significantly at 12, 24 and 48 hours zinc chloride treatment. The GPT activity showed decrease (83% and 64%) at 6 and 12 hours while LDH activity was inhibited (56%) only at 6 hours and showed recovery in remaining experimental period. Glycogen content increased (54% and 106%) while glucose showed significant decrease (43% and 45%) at 24 and 48 hours zinc exposure. RNA content increased 32%, 17% and 24% at 12, 24 and 48 hours treatments, respectively. In long-term experiment all the enzyme activities remained unchanged following zinc treatment, except LDH activity which was decreased (70 and 62%) at 1 and 2 week treatments. Prominent decrease (27, 24 and 38% at 1, 2 and 3, weeks) in glycogen content and increase (37 and 41% at 1 and 2 weeks, respectively) in glucose content was also observed. Free amino acids showed 56, 34 and 41% increase at 2,3 and 4 week treatments. Hepatic proteins also showed slight alterations.

Key words: Zinc, heavy metal, toxicity, fish, biochemical changes, liver.

INTRODUCTION

Due to unplanned and indiscrminate industrial waste disposal, the various effluents as bye-products, contaminated with heavy metals, are gradually proving to be a great nuisance for the terrestrial and aquatic ecosystems (Azad *et al*., 1984; Honda and Nogawa, 1987; Monkiewilz *et al*., 1987). Heavy metals constitute a very significant proportion of this waste effluent (Duffus, 1981; Ajmal *et al*., 1985). The toxicity of heavy metals on various components of our environment is well rocognised (Babich *et al.,* 1986; Hodson, 1988; Spierenburg, 1989). Extensive studies have been undertaken in different regions of the world which indicate the accumulation of large quantities of these metals in air, soil, water and animal tissues, especially in liver, kidney, muscle and some other tissues where they produce harmful effects (Ramamoorthy and Blumhagen, 1984; Ajmal *et al*., 1985; Arnac and Lassus, 1985; Beyar *et al*., 1985; Mason and MacDonald, 1988). These effects are more prominent in heavily industrialized urban areas. Automobile exhaust and burning of fossil fuel further increase this pollution (EPA, 1982; Yong-Luan *et al.,* 1985; Agarwal, 1991).

Large amounts (if accumulate) produced harmful effects in liver, muscle, kidney and other tissues of animals, which in turn disturbs the metabolism and hampers development and growth of fish (Speher, 1976; Anadon *et al*., 1984; Kadiiska *et al*., 1985; Lui, 1987; Ali *et al.,* 1988). These heavy metal residues render the fish muscle proteins unfit for human consumption. This pollution is also toxic to fish food because it disturbs the quality and quantity of food available to fish in aquatic environment

(Jackim *et al.*, 1970; Lalande and Pinel-Alloul., 1984; Ghore *et al.*, 1985; Shakoori and Ali, 1986, 1987; Iqbal, 1988).

Several reports exist in literature regarding the pathologies produced by zinc pollution in different organs of the fish (Cairns Jr. *et al.*,1971; Tort *et al.*, 1984, 1984; Kumari and Banerjee, 1986; Gautam and Agrawal, 1987). Sastry *et al.* (1987) studied the effect of zinc and cadmium on intestinal absorption of xylose and tryptophan in the freshwater teleost fish. Liver and kidney PBG synthetases are inhibited by zinc, magnesium and lead (Gonzalez *et al.*, 1987). In another report from this laboratory (Ali *et al.* 1988) biochemical effects of zinc on muscle have been reported, which showed significant increase in CPK activity while GOT activity, proteins and FAA decreased considerably.

In the present investigation, an attempt has been made to study some biochemical pathologies produced by zinc on fish liver which has an important role in the body as far as the metabolic regulation is concerned.

MATERIALS AND METHODS

Experimental animals and their maintenance

Cyprinus carpio, a common freshwater fish, was maintained in the round fibreglass aquaria (2x4 ft.) in small groups with constant aeration at 25.0 $\pm$ 1.0 °C. Before starting actual experiment, the animals were acclimatized for one week. During the experiment the fishes were provided with the feed (50g/day/aquarium) prepared by mixing rice polish and corn flour (9:1).

Heavy metal administration

Zinc chloride was administered to fish in two experimental aquaria (each with 32 fish and 75 litres of water) @ 50 μg/ml. In one aquarium, fish were maintained in zinc chloride treated water for 48 hours (short term experiment), while in another aquarium, fish were exposed to the same treatment for 4 weeks (long term experiment). Another two aquaria, with 32 fish and 75 litres of water, were used for control experiment. The water in the aquaria was replaced after every 48 hours and fresh dose of zinc chloride was added in long term experiment. Four control and four zinc chloride treated fishes were taken out at 6,12, 24 and 48 hour intervals in the short term experiment, and at 1,2,3 and 4 weeks duration in the long term experiment. The fishes were quickly knocked down, dissected, their livers taken out and stored immediately at -20 °C until further analysis.

Biochemical analyses

The details of liver processing for different biochemical analyses, have already been described elsewhere Ali *et al.*, 1988). The saline extract of liver was analysed for alkaline phosphatase (AP: orthophosphoric monoester phosphorylase, EC. 3.1.3.1.) activity according to Bessey *et al.* (1946); acid phosphatase (AcP: orthophosphoric

monoester phosphorylase, EC. 3.1.3.2) activity according to Andersch and Szcyphinski (1947); glutamate oxaloacetate transaminase (GOT: L- aspartate 2- oxoglutarate amino transferase, EC. 2.6.1.1) and glutamate pyruvate transaminase (GPT: L-alanine 2-oxoglutarate amino transferase, EC. 2.6.1.2.) activities according to Reitman and Frankel (1957) and lactate dehydrogenase (LDH: L- lactate- NAD^+ oxidoreductase, EC. 1.1.1.27) activity according to Cabaud and Wroblewski (1958).

The liver extract was further analysed for concentrations of various metabolites such as glycogen according to Shibko *et al.* (1967); glucose according to o-Toluidine method of Hartel *et al.* (1969); soluble and total proteins according to Lowry *et al.* (1951) and free amino acids (FAA) according to Moore and Stein (1954). DNA and RNA contents were extracted according to Shakoori and Ahmad (1973) and estimated according to Schmidt and Thannhauser procedure as described by Schneider (1957).

RESULTS

Zinc chloride treatment for 48 hours produced prominent inhibition of various enzyme activities in fish liver (Table I, Fig.1). Hepatic AP activity decreased quite significantly (42%, 36% and 55% at 12, 24 and 48 hours treatments, respectively) in short-term experiment. Similar decrease (50%, 41% and 43% at the same duration) was also found in AcP activity. The fall of activity in case of GOT was 48%, 23% and 42.5% at 12, 24 and 48 hours, while in GPT it was 83% and 64% during initial intoxication period *i.e.* 6 and 12 hours and 56% in case of LDH at 6 hour duration only. The liver showed significant glycogenesis (54% and 106%) with the simultaneous depletion (44% and 45%) of glucose content at 24 and 48 hours of zinc exposure. The soluble and total proteins did not indicate any severe effect, except 47% significant decline in total protein contents at the end (48 hours treatment) of the experiment. The FAA although, exhibited quite prominent increasing trend but this increase (86%) was significant only at 6 hour heavy metal intoxication. Amongst nucleic acids, DNA remain unchanged while RNA showed 32.5%, 16.5% and 24% rise at 12, 24 and 48 hours of zinc treatment.

In long-term zinc chloride administration, most of the tested hepatic enzyme activities (AP, AcP, GOT and GPT) behaved normally, except LDH activity which was significantly inhibited (69.5% and 62%) during first and second week and showed gradual recovery (36% and 28% non-significant decrease) during remaining two weeks experimental period (Table II, Fig. 2). The glycogen and glucose, both showed opposite trends when compared with short-term study. Prominent glycogenolysis (27%, 24%, 38% and 29% on 1st, 2nd, 3rd and 4th week, respectively) was observed during long-term zinc treatment study which was significant upto 3rd week. Hepatic glucose content increased by 37% and 44% on 1st and 2nd week of regular zinc exposure to fish. The FAA content showed almost similar changes, as found in short-term experiment, except that in this study the decrease (56%, 34% and 41.5% during last 3 weeks, respectively) was statistically quite significant. No significant change was found in the soluble proteins and nucleic acid (DNA and RNA) contents during this 4 weeks zinc treatment study on fish, *Cyprinus carpio*.

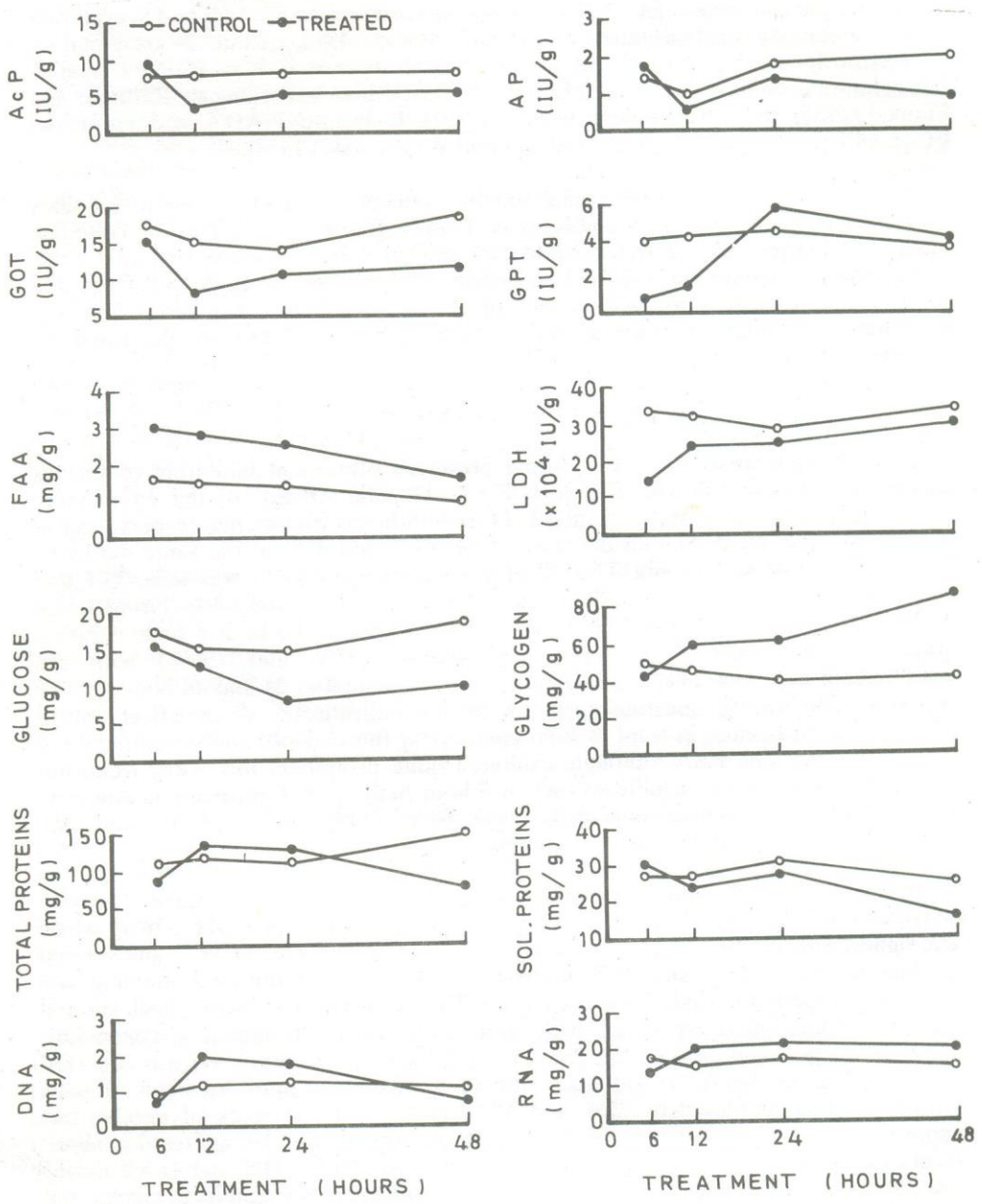

Fig. 1. Effect of zinc chloride (50μg) administered for 48 hours on some hepatic enzymes and metabolites of common carp, *Cyprinus carpio*.

Table I: Percent increase (+) or decrease (-) in various hepatic biochemical components of common carp, *Cyprinus carpio* expored to zinc chloride (50 μg /ml) for the total period of 48 hours.

Parameters[a]	Zinc chloride treatment (Hours) 6 (n=8)	12 (n=8)	24 (n=8)	48 (n=8)
AP	+4.19	-42.45*	-36.03*	-54.95*
AcP	+7.31	-50.00*	-41.04*	-43.30*
GOT	-14.59	-48.36*	-23.00	-42.47*
GPT	-83.13*	-64.25**	+25.71	+2.97
LDH	-56.14*	-29.17	-14.56	-9.30
Glycogen	-20.74	+29.93	+54.11**	+106.19***
Glucose	-10.63	-15.49	-42.68**	-44.96***
Soluble proteins	+3.80	-5.04	-4.88	-35.12**
Total proteins	-19.30	+3.45	+8.41	-46.60**
FAA	+85.63*	+78.79	+75.95	+52.60
DNA	-4.35	+61.98*	+32.82	-27.10
RNA	-4.70	+32.45*	+16.51*	+24.07*

*P<0.05, **P<0.01, ***P<0.001 (Student's 't' test)

Abbreviations used: AP, alkaline phosphatase; AcP, acid phosphatase; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; LDH, lactate dehydrogenase; FAA, free amino acids.

TABLE II: Percent increase (+) or decrease (-) in various hepatic biochemical components of common carp, *Cyprinus carpio* exposed to zinc chloride (50 μg/ml) for 4 week duration.

Parameters	Zinc chloride treatment (Weeks) 1	2	3	4
AP	-8.51	+6.71	+20.13	+0.71
AcP	-19.83	-17.89	-18.02	-18.60
GOT	-8.55	-10.08	+17.56	+2.98
GPT	-12.74	-20.61*	-6.44	-9.78
LDH	-69.51*	-61.69*	-36.07	-27.72
Glycogen	-26.84*	-24.19*	-37.83**	-28.82
Glucose	+36.88***	+43.81**	+5.83	-4.26
Soluble Proteins	-24.61	+7.33	+14.52	-6.26
Total Proteins	+9.56	+38.63*	-4.08	-12.66
FAA	+2.65	+56.14**	+33.63*	+41.44*
DNA	-5.96	-4.81	-22.56	+20.62
RNA	+2.73	+11.10	-3.82	+7.21

[a]For abbreviations, see Table I.

*P<0.05, **P<0.01, ***P<0.001 (Students's 't' test)

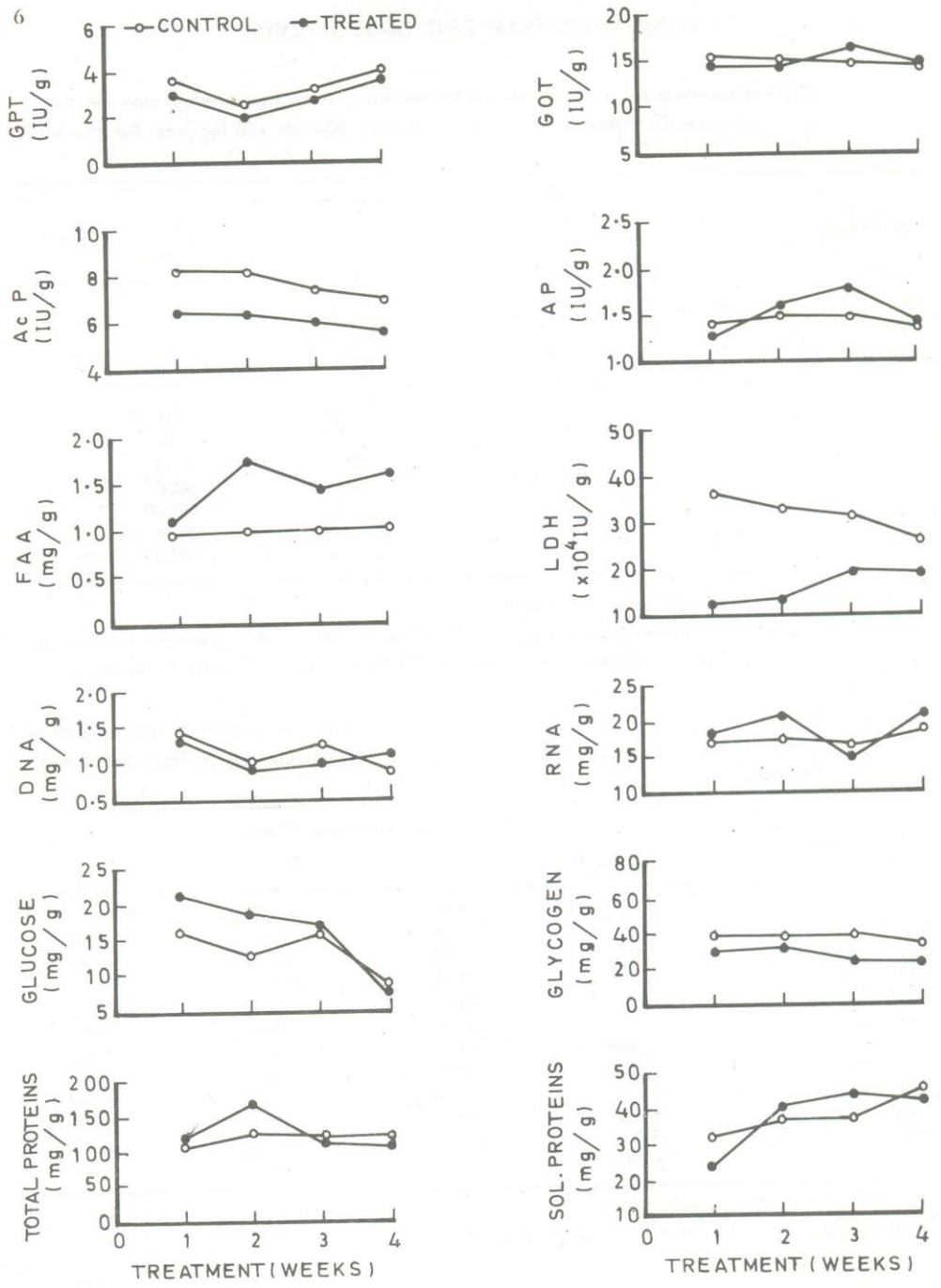

Fig. 2. Effect of zinc chloride (50μg) administered for 4 weeks on some hepatic enzymes and metabolites of common carp, *Cyprinus carpio*.

DISCUSSION

Since liver is the centre of metabloic activity, all toxic compounds are likely to be metabolized in this organ. The various hepatic enzymes are prone to toxic effects and can best be used as indicators of sublethal exposure of fish to toxic metals (Jackim *et al*., 1970).

Almost all the enzyme activities showed inhibition after treatment of zinc chloride for 48 hours in short-term experiment and for 4 weeks in long-term experiment. However, this inhibition was greatly reduced to non-significant level, except LDH in long-term experiment and significant changes were mostly observed in short-term experiment when compared to other treatment.

Both hepatic phosphatase (AP and AcP) activities decreased significantly in short-term experiment from 12 hours treatment. AcP is an important lysosomal enzyme while AP is found mostly in bones, and in the lining cells of the bile canaliculi. These both enzymes are pH-dependent and require acidic and basic pH, respectively. The zinc treatment may cause alteration in ionic balance with the resultant decrease in avtivities as these both reactions require metal ions as co-factors. Similar decrease in AP and AcP activities has also been shown in liver and serum of fish, *Clarias lazera* and *Talapia zilli* after zinc treatment (Hilmy *et al*., 1987). In long-term zinc treatment, both phosphatase activities showed recovery which may be due to induction of body's defence.

The both hepatic transaminase activities (GOT and GPT) decreased quite prominently in short-term zinc treatment while almost no change was found in long-term study. These both enzymes help in gluconeogenesis and decrease in their activity, possibly inhibit the transamination process required for regeneration of glucose from non glucose sources in the body. GPT and AP both are liver function enzymes, and decrease in their activities indicates the abnormal function of this very important organ.

The LDH is mostly found in muscle and liver tissues. This enzyme catalyse the reversable oxidation of lactate into pyruvate and has a key role in stimulating the gluconeogenesis on one side and citric acid cycle on other side. The decrease in its activity may directly interfere with glucose synthesis (gluconeogenesis) and also its oxidation through citric acid cycle, and lead to accumulation of lactic acid in muscle which is toxic in greater amounts. Tort *et al*.(1984, 1985) determined the gill metabolism after chronic and subacute zinc treatment to fish, for short and long-term treatments. It was found that zinc treatment increase the lactate concentration while lower the ATP contents of the gills which indicates inhibition of citric acid cycle. No significant changes were observed in ATP and lactate contents in long-term zinc exposure which suggests the induction of recovery processes and compensatory mechanisms in the body in long term treatment. The inhibition of both transaminase (GOT, GPT) activities also support these findings, because in the presence of greater amounts of lactate there is no need of its further synthesis through transamination. Similar studies with lead, cadmium and mercury on the fish, *Cirrhina mrigala* revealed increased hepatic enzyme activities (like AP, GOT, GPT and LDH) during short and

long term treatments (Shakoori and Ali, 1986, 1987; Iqbal, 1988).

The hepaic glycogen content did not changed until 24 hours of zinc exposure while increased, almost two fold, at the end of short-term treatment. This condition, most probably, developed due to the induction of glycogenesis from glucose which is not being utilized during inhibition of different enzyme activities required for glucose oxidation. Zinc chloride severely effected the glycogen contents in 4 weeks study which was apparent by significant decrease in its concentration in all treatments from Ist to 4th week, which may be due to the increased glycogenolysis or decreased gluconeogenesis. However this thing needs further experimentation that which mechansm is actually responsible for this alteration. The greater possibility is that former mechanism is involved in this case which it is also confirmed by the increase in glucose contents in this long-term study, while reverse was the case in short-term zinc chloride treatment.

The soluble proteins mostly remained unchanged in both studies, except at the end of short-term experiment (48 hour treatment) and at initial period of long term study (1st week) which showed non-significant decrease. Total proteins were also slightly effected with zinc treatment, which exhibited irregular pattern in both experiments. Significant reduction was observed at 48 hours in short-term treatment while 38% increase was noticed at 2nd week treatment in long-term zinc exposure.

Free amino acids (FAA) remained uneffected in 48 hours zinc feeding study, except at 6 hours when 85% significant increase was found. The changes in other treatments (12, 24 and 48 hours) were statistically non- significant. However, in long-term study the FAA contents attained a definite pattern, and showed significant increase except at 1st week when this component remained at normal level. The increase in FAA at this level is not understandable. One possibility may be the protein hydrolysis, but we could not find any decrease in protein contents during this study period. Similarly in the conditions when glycogen is depleting with rise in glucose there is no need of greater mobalization of FAA contents. Perhaps zinc treatment produce some changes in the intestinal brush border of the fish which resulted in the increased intestinal absorption of FAA. Shakoori and Ali (1987) showed decreased FAA at 50 μg cadmium chloride/ml while increased at a dose of 100 μg/ml in the liver of fish, *Cirrhina mrigala.*

The hepatic DNA content remained unchanged while RNA content increased only at 12 and 24 hours in short-term treatment. Similar findings were also obtained in other studies reported from this laboratory with cadmium as heavy metal (Shakoori and Ali, 1987). This increase may be due to induction of drug metabolizing enzymes necessary for compensatory mechanism which is evident from long term studies where effect of zinc became dilute when compared to short term study. It is oberved that zinc and cadmium salts increase the activities of hepatic drug metabolizing enzymes such as the activity of benz-phetamine-*n*-demethylase. These metals also increase the contents of cytochrome P-450 and microsomal heam which are also important components of detoxification systems in animals. The heavy metal salts like zinc and cadmium increased the activity of amino-levulinic acid synthetase and

decreased the heam-oxygenase (Kadiiska, *et al.* 1985).

These findings suggest that although zinc is an essential micronutrient for animals but when present in toxic amounts in our environment, it induces various type of toxic effects in aquatic and terrestrial animals.

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