Bull. Environm. Contam. Toxicol. 20, 353-360 (1978)

Effect of Mercuric Chloride on the Digestive System of a Teleost fish, *Channa punctatus*

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Introduction

Heavy metals produce toxic effects on the tissues and alter the physiological functioning of various systems of animals. Mercury and copper are among the most toxic heavy metals to fishes. Toxicity of mercurial compounds to fishes has been reported by JONES (1939), GREESON (1970), and REHWOLDT et al. (1972). Mercury deposits in the tissues of fishes have been reported by SMITH et al. (1974) while LOCKHART et al. (1972) determined the elimination rate of methyl mercury in heavily contaminated Northern pike. HINTON et al. (1973) observed liver damage in the form of necrosis in portal areas and formation of connective tissue septa due to methyl mercury poisoning. LIN et al. (1975) studied toxic effects of methyl mercury on different ages of rats. CHANG et al. (1973) studied the histochemical changes in kidney, liver and brain of rat after chronic mercury intoxication.

Data regarding the response of teleost fishes to mercury intoxication have been limited to description of specific tissue pathology. The present communication deals with the alterations in the enzyme activities that accompany mercury intoxication in the different parts of the digestive system of a teleost fish, Channa punctatus, after treatment with LC_{50} for 96 hours.

0007-4861/78/0020-0353 $01.60

Materials and Methods

Living fishes were collected from local fresh water sources and maintained in laboratory aquaria. Specimens weighing 60-70 g each were selected and prior to experimentation. They were acclimatized to the laboratory conditions for 3 days. Preliminary bioassays conducted in the laboratory under static conditions have shown that the LC_{50} for 96 hours is 1.8 mg/liter. The first group of 15 fishes were treated with this concentration for 96 hours while a second group of 15 fishes maintained in mercury-free tap water served as controls. 10/ (W/V) homogenates of stomach, intestine, pyloric caeca and liver were prepared in 0.25M sucrose solution in cold. The homogenates were centrifuged for 20 minutes at 1000 g and the clear supernatant fluids were used as the source of enzymes 0.016M sodium β-glycerophosphate was used as the substrate at pH 5.0 and 9.3 for acid and alkaline phosphatase, respectively. The enzyme activity was estimated according to the method of BODANSKY (1933). For the estimation of glucose-6-phosphatase activity, 0.01M glucose-6-phosphate solution was incubated for 15 minutes at pH 6.5. The method of SWANSON (1965) was followed. Amylase was estimated according to BERNFIELD (1955). The substrate was 0.5M starch solution and the incubation period was 1 hour. ANSON's (1939) method was adopted for the estimation of the activities of trypsin and pepsin. Peptidases were determined by the method of SMITH (1950). After incubation with substrates the individual amino acids in 10 µl of the incubation mixture were separated by paper chromatography.

The intensity of the coloured spots of glycine developed with ninhydrin was scanned in a Systronyx densitometer.

Results and Discussion

The results of the experiments conducted are presented in tables 1 and 2.

The present work has been undertaken to observe whether mercuric chloride produces any alterations in the activity of digestive enzymes. Mercury compounds are known to produce severe damage in liver and kidney of fishes but very few references are available on the effects in the digestive system, though some amount of mercury enters the digestive system through food. In the present study, confined to a short term 96 hour LC_{50} treatment, it has been observed that mercuric chloride does not produce any significant alteration in the activity of digestive enzymes. Significant inhibition occurred only in the activities of the hepatic phosphatases showing that the physiological functioning of this organ is affected. The decrease in the alkaline phosphatase activity may be due to either the direct action of mercury or the toxic effects it produces in the liver. HINTON et al. (1973) and KENDALL (1975) reported similar inhibition in the hepatic alkaline phosphatase activity in fishes. Feeding methyl mercury to pike is reported to produce toxic changes in liver. Cellular damage is usually accompanied with an increase in acid phosphatase activity. But in the present study the activity is inhibited which again may be due

TABLE 1

The activities of phosphatases in experimental and control fishes[a]

Enzyme	Tissue	Number of experiments conducted	Control	Experimental	Level of significance
Alkaline phosphatase	Liver	3	0.0494 ± 0.00073	0.0445 ± 0.00020	8.59 (+)[b]
	Stomach	3	0.0621 ± 0.00106	0.0480 ± 0.00100	12.81 (+)
	Intestine	3	0.0398 ± 0.00020	0.0470 ± 0.00023	27.69 (+)
	Pyloric caeca	3	0.0360 ± 0.00076	0.0568 ± 0.00090	21.66 (+)
Acid phosphatase	Liver	3	0.0458 ± 0.00073	0.0425 ± 0.00066	4.125 (+)
	Stomach	3	0.0545 ± 0.00093	0.0531 ± 0.00206	1.00 (-)
	Intestine	3	0.0588 ± 0.00150	0.0544 ± 0.00073	3.38 (+)
	Pyloric caeca	3	0.0577 ± 0.00063	0.0573 ± 0.00040	0.625 (-)
Glucose-6-phosphatase	Liver	3	0.0566 ± 0.00141	0.0571 ± 0.00066	1.00 (-)
	Stomach	3	0.0729 ± 0.00130	0.0674 ± 0.00078	4.58 (+)
	Intestine	3	0.0626 ± 0.00085	0.0590 ± 0.00077	3.87 (+)
	Pyloric caeca	3	0.0580 ± 0.00210	0.0552 ± 0.00066	1.64 (-)

a. Activity is expressed in mg. of inorganic phosphate liberated per mg of tissue protein per hour at 37°C (Mean ± S.E.).

b. (+) indicates statistically significant difference from control values at 95 percent confidence interval.

TABLE 2

The activities of digestive enzymes in experimental and control fishes[a]

Enzymes	Tissue	Number of experiments conducted	Control	Experimental	Level of significance
Amylase (mg. maltose/ mg. protein/ hour)	Liver	3	0.187 ± 0.0083	0.196 ± 0.0036	1.232(-)
	Stomach	3	0.066 ± 0.0007	0.079 ± 0.0020	7.64(+)b
	Intestine	3	0.081 ± 0.0010	0.093 ± 0.0020	7.05(+)
	Pyloric caeca	3	0.132 ± 0.0069	0.077 ± 0.0056	7.63(+)
Trypsin (mg. tyrosine/ mg. protein/ hour)	Intestine	3	0.2603 ± 0.0700	0.4656 ± 0.0270	3.360(+)
	Pyloric caeca	3	0.2551 ± 0.0164	0.3780 ± 0.0380	3.604(+)
Pepsin (mg. tyrosine/ mg. protein/ hour)	Stomach	3	0.4172 ± 0.6330	0.6096 ± 0.4080	3.133(+)
Triglycine Triptidase (mg. glycine/ mg. protein/ hour)	Liver	3	0.152 ± 0.079	0.150 ± 0.0107	0.040(-)
	Intestine	3	0.156 ± 0.019	0.160 ± 0.0050	0.317(-)
	Pyloric caeca	3	0.174 ± 0.0069	0.172 ± 0.0036	0.210(-)
Glycyl-glycine dipeptidase (mg. glycine/ mg. protein/ hour)	Intestine	3	0.172 ± 0.0057	0.171 ± 0.0011	0.188(-)
	Pyloric caeca	3	0.196 ± 0.0063	0.183 ± 0.0094	1.413(-)
Carnosinase (mg. glycine/ mg. protein/ hour)	Intestine	3	0.143 ± 0.0070	0.150 ± 0.0079	1.02(-)
	Pyloric caeca	3	0.152 ± 0.0420	0.155 ± 0.0094	0.088(-)

a. Values are Mean ± S.E.

b. (+) indicates statistically significant differences from control values at 95 percent confidence interval.

to direct action of mercury. In contrast to liver, intestine and pyloric caeca show a slight elevation in the acid and alkaline phosphatase activities. CHANDRA and IMAM (1973) have also reported an increase in acid phosphatase activity in the intestinal mucosa of animals treated with manganese, which according to ZONEK et al. (1966) is due to increased pinocytosis. The decrease in the glucose-6-phosphatase activity may be attributed to the disturbances in the general metabolism of the cell due to mitochondrial damage.

Amylase and proteases have shown an increase in activity which reveals that the digestive system is not affected by mercury treatment. In fishes, the pancreas is diffused and in this condition also it secretes some digestive enzymes. Under mercury intoxication, it might be possible that the pancreatic enzymes like trypsin and tripeptidase are released into the gut. The intestinal enzymes like dipeptedases are either unaltered or show a slight inhibition in activity which is statistically insignificant.

Summary

The effect of 1.8 mg /liter (LC_{50}) of mercuric chloride exposure on the activities of alkaline phosphatase, acid phosphatase, glucose-6-phosphatase, amylase, pepsin, trypsin, tripeptidase glycyl-glycine dipeptidase and carnosinase has been examined in Channa punctatus. The three phosphatases have been inhibited in the liver but showed an increase in activity in the intestine and pyloric caeca. Amylase, pepsin and trypsin have also shown a slight increase in activity. There has been no significant

alteration in the activities of the peptidases. The results show that mercury inhibits the activities of phosphatases in the liver but has no significant effect on the digestive enzymes within the experimental period of 96 hours.

Acknowledgement

The authors are thankful to Dr. V.P. Agrawal, Principal, D.A.V. College, Muzaffarnagar for criticism and help. The financial assistance by the University Grants Commission, New Delhi is gratefully acknowledged.

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