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Effects of Liv.52 Against Cadmium Chloride-Induced Changes on the Activity of Enzyme and Mucosubstances in the Intestine of *Mystus Tengara* (HAM)

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ABSTRACT

The histochemical findings in the freshwater fish M. tengara indicate that cadmium (Cd) inhibits the activity of intestinal alkaline phosphatase, glucose-6-phosphatase and 5' nucleotidase and induces the excessive secretion of acid mucopolysaccharides. On the other hand, Liv.52 provides protection against the toxic action of Cd and helps in regulating the secretary activity of enzymes.

INTRODUCTION

Cadmium has been reported to inhibit enzymes in various animal tissues (Lipkan 1970, Cross *et al.* 1970). Both reduction and an increase in enzyme activities in mammalian organs due to Cd have been reviewed (Valle and Ulmer 1972). Biochemical studies on the alterations in enzyme activities in fish due to Cd alone (Kumada *et al.*, 1980, Sastry and Subhadra 1985, Kothari and Saxena 1988) and Cd and other heavy metals (Jackim, 1973) have also been conducted in the past. Attempts have been made to find out the effectiveness of an indigenous drug, Liv.52 on enzyme activity in mammalian systems against a large variety of toxins (Agrawal *et al.,* 1982, Bardhan *et al.,* 1985). Similar studies on the protection against toxicity in fish organs are wanting.

It was therefore decided to study the protective role of Liv.52 against heavy metal toxicity. The present study deals with the toxic effects of $CdCl_2$ and the effectiveness of Liv.52 on the feeding and secretory activity of enzymes and mucosubstances in the intestine of a freshwater fish *Mystus tengara* (Ham.), histochemically.

MATERIALS AND METHODS

Healthy specimens of catfish, *Mystus tengara* were procured from the local fresh-water bodies and were acclimatized to laboratory conditions for one week. Water conditions were as follows: DO 6.8 ppm hardness (as $CaCO_3$) 116 mg/l, alkalinity 168 mg/l, chloride 41 mg/l and pH 6. The average water temperature was 18^{o}C.

The experiment was conducted with a sublethal concentration of $CdCl_2$ for 30 days. The LD_{50} (96 h value) of $CdCl_2$ to *M. tengara* was found to be 116 mg/l. Protection against metal toxicity was tried with an indigenous Ayurvedic remedy Liv.52 (The Himalaya Drug Co., Bombay).

The acclimatized animals were divided into five groups of 15 fish each and were kept in five glass aquaria, each containing 10 l of dechlorinated tap water. Fish of all the groups were fed daily with dried and chopped prawns (300 mg/aquarium). Food was given in the form of balls prepared by mixing it with liquid paraffin. The daily dose of Liv.52 was 7 mg/aquarium mixed with the food and paraffin to prepare the balls.

In the present investigation five groups of the fish were maintained as shown in Table 1.

Table 1: Experimental groups (30 days' duration)

Group I	Control fed on normal food (No Cd and no Liv.52)
Group II	Treated with Cd and fed on normal food (No Liv.52)
Group III	Treated with Cd and fed on food containing drug (Poison + Liv.52)
Group IV	First 15 days: Treated with Cd and fed on normal food (No Liv.52) Next 15 days: Treated with Liv.52 (No poison)
Group V	Treated with only Liv.52 (NO poison)

No artificial aeration was done in aquaria water during the experimental period. For feeding behavior studies, observations were made on the feeding intensity, utilization of food and the time required for the consumption of food.

For the studies on enzymes and mucosubstances, fish from each group were dissected on the 31st day and the intestine was separated into anterior and posterior parts, cleaned and cut into pieces of 1 cm length. Histochemical techniques as described by Pearse (1968 and 1972) were applied for the localization of alkaline phosphatase (ALP), glucose-6-phosphatase (G-6-P), 5'-nucleotidase (5'-NTD) and acid mucopolysaccharides (AMP).

To assess the alterations in the activity of enzymes and mucus and to find out the protective role of Liv.52, sections of intestine from each group were compared on the basis of staining intensities.

RESULTS

Observations on the feeding behaviour of *M. tengara* in different experimental groups indicated that the given food was not consumed completely in Cd-contaminated water (Groups II, III and IV). On the other hand, in the control groups the food was completely consumed within 15 minutes and in Group V (administered only Liv.52) in just five minutes. This indicates that Cd decreased the appetite whereas Liv.52 increased the appetite of the fish.

Alkaline phosphatase: As compared to the control fish (Fig. 1), the activity of ALP was inhibited in the Cd-poisoned fish intestine (Group II). Enhanced phosphatase activity was noticed following Liv.52 therapy in Groups III and IV (Fig. 2). However, a decline in enzyme activity was found in animals belonging to Group V. Both proximal and distal parts of intestine reacted in a similar way to the poison and Liv.52. In general, enzyme concentration was low in the distal part than in the proximal part. Results of staining intensities of ALP in the intestine are tabulated in Table 2.

Table 2: Alkaline phosphatase activity in different experimental groups of *M. tengara*

Tissue	Anterior intestine					Posterior intestine				
	Groups					Groups				
	I	II	III	IV	V	I	II	III	IV	V
Mucosa	++	–	+	+	–	+	+–	+	+	+–
Goblet cells	–	–	–	–	–	–	–	–	–	–
Col. epithelium	+	+	++	+++	+–	–	–	+	+	–
Submucosa	+–	+–	+	+	–	–	–	+	–	–
C.M.F.	–	–	+	+–	–	–	–	–	–	–
L.M.F.	–	–	+	+–	–	–	–	–	–	–
Serosa	–	–	+	+–	–	–	–	–	–	–

+– Weak positive activity
\+ Positive activity
++ Strong positive activity
+++ Very strong positive activity
– Negative activity

Glucose-6 phosphatase: Significant reduction in G-6-P was noticed in the anterior intestine of fish belonging to Groups II and III with a total loss in Group IV. Weak positive activity of enzyme was demonstrated in the fish of Group V, fed with only Liv.52 (Figs. 3 and 4). In the posterior intestine total loss of G-6-P was recorded in Groups II, III and IV, whereas it was almost similar in Groups I and V. Details of staining intensities of G-6-P are given in Table 3.

Table 3: Glucose-6-phosphatase activity in different experimental groups of *M. tengara*

Tissue	Anterior intestine					Posterior intestine				
	Groups					Groups				
	I	II	III	IV	V	I	II	III	IV	V
Mucosa	+	–	–	–	+–	+–	–	–	–	–
Goblet cells	–	–	–	–	–	–	–	–	–	–
Col. epithelium	++	+–	+–	–	+–	+–	–	–	–	+–
Submucosa	+	+–	+–	–	+–	+–	–	–	–	+–
C.M.F.	+–	–	–	–	+–	+–	–	–	–	+–
L.M.F.	+–	–	–	–	+–	+–	–	–	–	+–
Serosa	+	–	–	–	+–	+–	–	–	–	+–

+– Weak positive activity
+ Positive activity
++ Strong positive activity
– Negative activity

5'-Nucleotidase: Complete loss of 5'-NTD was noticed in Group II due to metal poisoning. Gradual increase in enzyme activity was observed in the intestine in Groups III and IV. In intestine of the fish kept in Cd-free water and fed on Liv.52 containing food, 5'-NTD activity was significantly enhanced and was even higher than in those of control fish (Figs. 5 and 6). Posterior intestine gave negative reaction for 5'-NTD in all the five experimental groups. Results of 5'-NTD staining are tabulated in Table 4.

Table 4: 5'-nucleotidase activity in different groups of *M. tengara*

Tissue	Anterior intestine					Posterior intestine				
	Groups					Groups				
	I	II	III	IV	V	I	II	III	IV	V
Mucosa	–	–	–	–	++	–	–	–	–	–
Goblet cells	–	–	–	–	++	–	–	–	–	–
Col. epithelium	++	–	+–	+	+–	–	–	–	–	–
Submucosa	++	–	–	+	++	–	–	–	–	–
C.M.F.	–	–	–	–	–	–	–	–	–	–
L.M.F.	–	–	–	–	+	–	–	–	–	–
Serosa	–	–	–	–	+–	–	–	–	–	–

+– Weak positive activity
+ Positive activity
++ Strong positive activity
– Negative activity

Table 5: Acid mucopolysaccharides secretion in different groups of *M. tengara*

Tissue	Anterior intestine					Posterior intestine				
	Groups					Groups				
	I	II	III	IV	V	I	II	III	IV	V
Mucosa	+–	++	++	++	+–	+–	++	++	++	++
Goblet cells	–	++	++	++	–	–	+–	++	++	+++
Col. epithelium	–	–	–	–	–	–	–	–	–	–
Submucosa	–	–	–	–	–	–	–	–	–	–
C.M.F.	–	–	–	–	–	–	–	–	–	–
L.M.F.	–	–	–	–	–	–	–	–	–	–
Serosa	–	–	–	–	–	–	–	–	–	–

+– Weak positive activity
++ Strong positive staining
+++ Very strong positive activity
– Negative activity

Acid mucopolysaccharides: In the intestine of Cd-exposed fish (Groups II, III and IV) section of AMP was greatly enhanced as compared to the control fish in the anterior intestine of fish. AMP secretion was similar in Groups I and V, while in the posterior intestine, AMP deposition was found to be maximum in Group V (Figs. 7 and 8). The distribution and concentration of AMP are shown in Table 5.

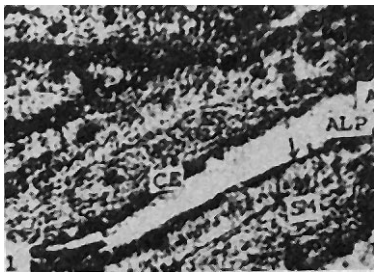

Fig. 1: Section of anterior intestine of control fish showing activity of alkaline phosphatase (X 200).

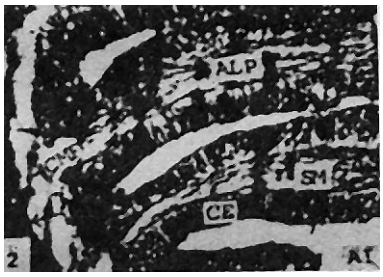

Fig. 2: Section of anterior intestine of Cd + drug (Group III) treated fish showing enhanced alkaline phosphatase activity (X 200).

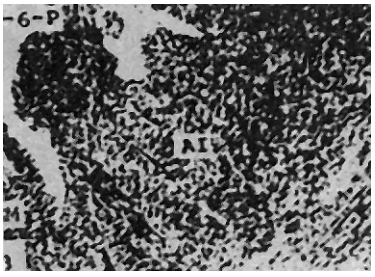

Fig. 3: Section of anterior intestine of control fish showing distribution of glucose-6-phosphatase (X 200)

Fig. 4: Section of anterior intestine of Cd exposed fish (Group II) indicating inhibited glucose-6-phosphtase activity

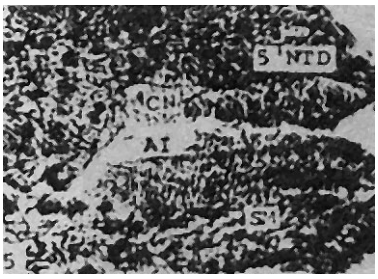

Fig. 5: Section of anterior intestine of control fish exhibiting 5'-nucleotidase activity (X 200)

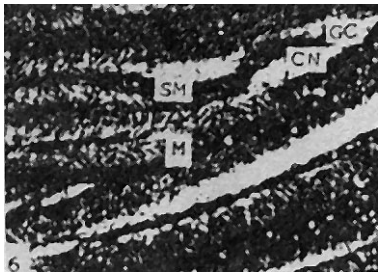

Fig. 6: Section of anterior intestine of group V (only drug) showing significantly enhanced 5'-nucleotidase activity (X 200)

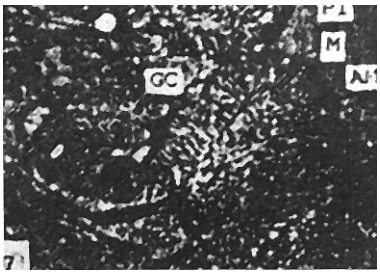

Fig. 7: Section of posterior intestine of control fish showing distribution of acid mucopolysaccharides (X 200)

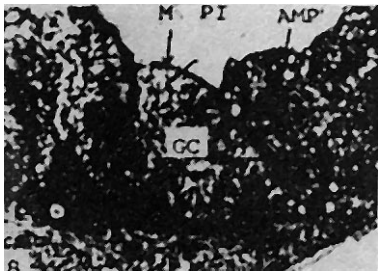

Fig. 8: Section of posterior intestine of Cd exposed fish (Group III) showing excessive secretion of acid mucopolysaccharides (X 200)

Al = Anterior intestine
ALP = Alkaline phosphatase
AMP = Acid mucopolysaccharides
CE = Columnar epithelium
CMF = Circular muscular fibres
CN = Columnar nuclei
GC = Goblet cells
G-6-P = Glucose-6-phosphatase
M = Mucosa
Pl = Posterior intestine
SM = Submucosa

DISCUSSION

Passiveness and poor appetite due to Cd toxicity have been reported in zebra fish, *Brachydanio rerio* (Karlson-Norrgren *et al.*, 1985). During the present investigation also, it was found that the rate of food consumption in Cd-exposed fish was significantly reduced, whereas it was very much increased in the Liv.52 fed fish (Group V) causing a pseudostarved condition.

Liv.52 has already been known to cause an increase in food consumption and more efficient food utilization (Srinivasan and Balwani, 1968). Following the exposure to Cd, fish did not consume the given food. It is possible that the mucosal lining might have been injured (enteropathy) and as a consequence the usual digestion of food must have been affected. Liv.52 is known to repair cellular membranes of the liver of mammals damaged following exposure to CCl_4, C_2H_5OH, amyl alcohol, paracetamol and other toxins (Joglekar *et al.,* 1963, Joglekar and Balwani, 1967). It has

also been shown that Liv.52 prevents degeneration in human jejunum and causes regeneration of villi (Tripathi *et al.,* 1977). Hence, in the present case also it might have tried to preserve the structural integrity of the intestine which could synthesize some 5'-NTD, even in the presence of Cd which was totally lost following Cd-exposure. On the other hand, alkaline phosphatase, which is vital for normal functioning of intestine, and otherwise loses its activity following Cd-exposure, is maintained at optimal level with Liv.52. Even in the presence of Cd (Group III) Liv.52 could maintain ALP activity, which is inhibited following Cd exposure in *Mystus tengara*. The inhibitory effect of Cd on alkaline phosphatase (Venugopal and Luckey, 1975) and ATPase (Lipkan 1970, Cross *et al.,* 1970) is already known.

It was also found that in Group V, when the fish were administered only Liv.52 (in absence of Cd), a reduction in ALP and G-6-P occurred as compared to the control fish. It is probably so because the given food may not have been sufficient to be metabolized, as Liv.52 is known to enhance the ability of cells to digest more food (Srinivasan and Balwani, 1968).

Earlier biochemical studies also reported alterations in the activity of several enzymes due to toxicity of Cd and other heavy metals in fish. Jackim (1973) reported that induction of some liver enzymes is a common toxicological phenomenon and found an increase in enzyme activity in the liver of *Fundulus heteroclitus* due to heavy metals, including Cd. Inhibitory effect of Cd on liver and kidney enzymes in rainbow trout (Kumada *et al.,* 1980) and liver catalase activity in *Sarotherodon mossambicus* (Singh and Sivalingam, 1982) has been shown.

Enhanced secretion of mucus in Cd-exposed fish (Groups II, III and IV) seems to be a defensive mechanism against Cd toxicity. AMP is known to play a protective role against chemical and mechanical injuries in fish tissues (Kapoor *et al.,* 1975, Shaffi, 1980). Liv.52 does not seem to play any role in the secretion of AMP.

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