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Protective Role of Liv.52 Against Histological Damage due to $CdCl_2$ Toxicity in the Intestine of Teleost Fish

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ABSTRACT

Fresh water catfish Mystus tengara (Ham.) were experimentally exposed to sublethal concentrations of $CdCl_2$ for 30 days, to assess the structural changes in their intestines and the role of an indigenous drug Liv.52 in preventing such changes. The water quality and feeding behaviour of fish were also studied.

Cd poisoning resulted in cell death and necrosis in the columnar epithelial cells at the tips of the villi in the anterior and posterior intestine, respectively. Liv.52 was found to play a protective role against the structural damage in the Cd-exposed fish intestine, while its administration in the absence of Cd resulted in a very healthy and hyperactive condition of the intestinal tissues. Changes in the physico-chemical characteristic features of water were noticed due to Cd contamination. Behavioural studies revealed that Cd adversely affected the feeding behaviour of the fish, whereas Liv.52 greatly enhanced the feeding activity of the experimental fish indicating that Liv.52 provided protection to the fish intestine against $CdCl_2$ toxicity.

INTRODUCTION

Cadmium is a potentially hazardous pollutant in the environment. The methods for the passage of metal ions across the surface of intestinal and other biological membranes are well reviewed (Stein, 1967; Skoyma and Waldron, 1971). Cd toxicity in fish has been reported to depend on water quality criteria (Eisler, 1971; Slobe and Flook, 1975; Pascoe and Cram, 1977; Calamari *et al.,* 1980). Toxic effects of Cd with different concentrations and duration of exposure are well documented in teleosts (Nakamura, 1974; Voyer *et al.,* 1975; Dubale and Shah, 1979; Singh and Sivalingam, 1982; Stromberg *et al.,* 1983; Kothari and Saxena, 1988) and in other animals by Friberg, 1977. Chemical symptoms of Cd toxicity are well documented (Browning, 1969). Absorption and accumulation of Cd in the digestive tract have been described in fish (Edgren and Notter, 1989; Kumuda *et al.,* 1980) and in mammals (Venugopal and Luckey, 1975).

Studies on the protection of mammalian organs against a wide variety of toxicants with an indigenous drug Liv.52 have been conducted in the past (Rao, 1985; Prasad, 1975, 1976; Joglekar *et al.,* 1963; Patel and Sadra, 1963). Such protection studies in fish are wanting. Recently, Rathore and Rawat (1989) have reported that Liv.52 could protect against Cd-induced histological changes in mouse gut.

With this background the present study was undertaken to find out whether Liv.52 could provide protection to fish organs against $CdCl_2$ intoxication. The present paper deals with the histopathology and protective role of Liv.52 in the Cd-exposed intestine of fresh water teleost *Mystus tengara* (Ham.).

MATERIALS AND METHODS

Living and healthy specimens of *Mystus tengara* were collected from local fresh water sources and acclimatized to laboratory conditions for 7 days. The LD_{50} value (96 h) of $CdCl_2$ for this fish was determined to be 115 mg/l. The present investigation was carried out with 20 mg/l $CdCl_2$ for 30 days. No mortality occurred during this period.

For this experiment acclimatized fish were divided into 5 groups of 15 fish each and kept in glass aquariums each containing 10 litres of stored tap water. Fish in each aquarium were fed daily with dried and chopped prawns at the rate of 300 mg/day/aquarium. The food was mixed with liquid paraffin and formed into balls.

Protection by Liv.52 (The Himalaya Drug Company) against Cd toxicity was evaluated in this trial. The dose of Liv.52 was 7 mg/day/aquarium. It was mixed with the dry food and paraffin balls were prepared. Five groups of fish were maintained for this investigation as shown in Table 1.

Table 1: Experimental groups (30 days' duration)

Group I		Control group fed on normal food (No Cd or Liv.52)
Group II		Treated with Cd and fed on normal food (Cd)
Group III		Treated with Cd and fed on the food containing Liv.52 (Cd + Liv.52)
Group IV	A	Fish treated with Cd for the first 15 days and fed on normal food (Cd)
	B	For another 15 days fish were kept in Cd-free water and fed with food containing Liv.52 (Post Liv.52 therapy)
Group V		Treated with Liv.52 only.

The following studies were conducted:

1. *Water analysis*: Physico-chemical analysis was done for dissolved O_2, hardness, alkalinity, chloride contents, pH and temperature. The analysis was done as described in 'Chemical analysis of fresh water' and 'Standard methods for analysis of water' (1975).
2. *Behavioural studies*: Swimming and feeding activities of fish were observed.
3. *Histopathology*: For histopathological studies paraffin sections of 6 micron thickness were cut and double stained with haemotoxylin and eosin. A comparison of the intestinal sections from different groups was made, using a light microscope for histopathological studies.

OBSERVATIONS

Physico-chemical analysis of water

In the five experimental groups, dissolved O_2 in the water ranged between 5.2 to 6.8 mg/l, while hardness (as $CaCO_3$) and alkalinity were found ranging between 116 to 126 mg/l and 168 and 190 mg/l, respectively. Chloride contents in the water varied between 41.0-56.3 mg/l. Hydrogen ion concentration was found between 6.0 to 6.8, while the average temperature of the water during the experimental period was 18^{o}C. The average values for these water parameters in the five experimental groups are given in Table 2.

Table 2: Physico-chemical analysis of water in control and experimental groups (average values)						
Water parameters	Unit	Group I (Control)	Group II (Cd)	Group III (Cd + Liv.52)	Group IV (Cd + Liv.52 later)	Group V (Liv.52 only)
Dissolved O_2	mg/l	6.8	5.2	5.6	5.6	6.4
Hardness	mg/l	116	126	124	126	120
Alkalinity	mg/l	168	190	182	186	176
Chlorides	mg/l	41	56.3	53.1	53.1	45.9
pH	-	6	6.3	6.7	6.8	6.5
Temperature	^oC	18	18	18	18	18

Behavioural studies

Behavioural studies indicated that the fish of Groups I (Control) and V (Liv.52 only) were active with normal swimming movements, while those of Groups II (Cd), III (Cd + Liv.52), and IV (A) (Cd first) were passive with retarded swimming movements. Animals of Group IV (B) (Liv.52 later) gradually attained normal swimming behaviour. Fish of Group I (Control) with normal appetite consumed the given food within 15 minutes, while those of Group II (Cd), III (Cd + Liv.52) and IV (Cd first + Liv.52 later) could not consume food even in 24 hours. On the contrary, animals of Group V (Liv.52 only), exhibited significant increase in appetite and consumed the provided food within just 5 minutes.

Histopathology

The intestine of the fish was studied in both the anterior and posterior parts. A comparison of the intestinal sections from different groups was made to find out the histopathological effects of Cd and the role of Liv.52 against structural changes.

(a) *Anterior intestine*

Group I (Control) – Fish of this group exhibited normal structure of the intestine with long and tapering villi with tightly packed submucosal tissue (Fig. 1).

Group II (Cadmium) – Histology of the anterior intestine indicated broadening and flattening of the villi towards the tips. The area of each villus facing the lumen exhibited cell death, and the cells did not show distinct nuclei and cytoplasmic boundaries. Clear spaces were visualized towards the tips of the villi, due to shrinkage of submucosal tissue. The basement membrane was also found damaged and distorted (Fig. 2).

Group III (Cd + Liv.52) – Fish exposed to Cd were also administered Liv.52 along with food. Cd toxicity was found to be suppressed, as the degeneration was seen only in a few columnar cells. Broadening of the villi and shrinkage in the submucosa were not evident.

Group IV (Cd first and Liv.52 later) – In this group fish were first exposed to Cd for 15 days and then transferred to Cd-free water, and were administered Liv.52 with food for 15 days. The intestine of this group did not show any severe structural damage. However, distortion of

the basement membrane and slight shrinkage of the submucosa at the tips of the villi as a result of Cd poisoning were still evident (Fig. 3).

Group V (Liv.52 only) – In this group, the fish were given Liv.52 along with food. Histology of the intestine showed hyperactivity of the cells in the villi (elongated), which were found to be very well developed and healthy, and resulted in a reduction of the lumen (Fig. 4).

ANTERIOR INTESTINE

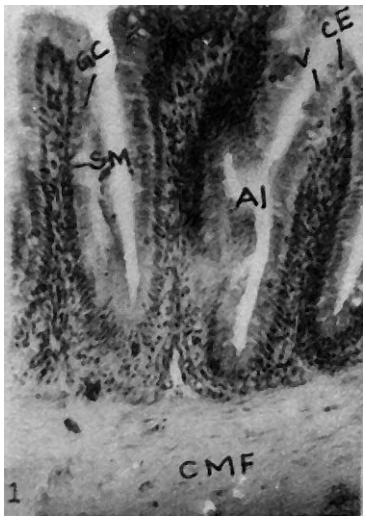

Fig. 1: Section showing histology of the anterior intestine of control fish (x 200).

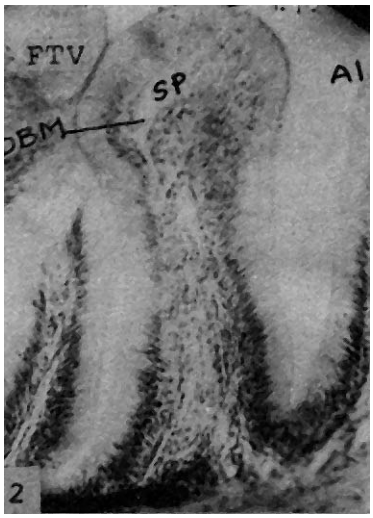

Fig. 2: Section of anterior intestine of Cd-exposed fish showing cell death in the columnar cells, distortion of basement membrane and shrinkage in the submucosa at the tip of the villi (x 200).

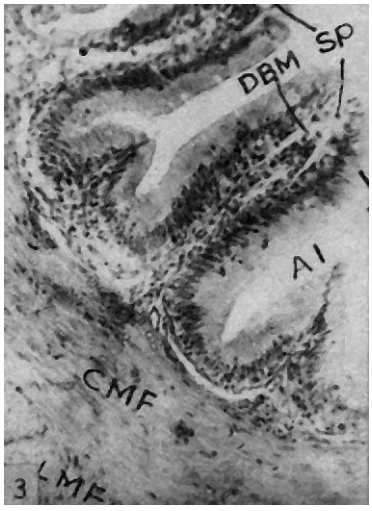

Fig. 3: Section of anterior intestine of Cd and drug-administered fish (15 days each), showing slightly degenerated basement membrane with narrow normal tips (x 200).

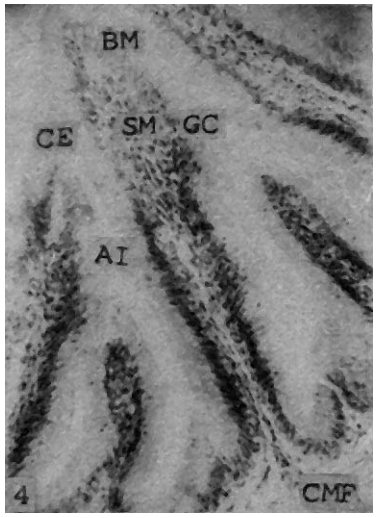

Fig. 4: Section of anterior intestine of Liv.52-administered fish showing hyperactivity of columnar cells with elongated villi (x 200).

(b) Posterior intestine

Group I (Control) – In the posterior intestine the musculature was thinner and the villi less tall than in the anterior intestine.

Group II (Cadmium) – Histology in fish following Cd exposure revealed that the height of the villi was significantly lower than in the controls. Most of the villi exhibited necrosis at the tips (Fig. 5).

Group III (Cd + Liv.52) – When fish were exposed to Cd and also given the drug along with food, their posterior intestine exhibited hyperactivity of cells in the villi. The villi were comparatively taller than those observed in Group II animals. No pathological changes were noticed (Fig. 6).

Group IV (Cd first and Liv.52 later) – In the intestine of these fish, the villi were longer and thinner compared to Group II, and revealed a structure similar to that observed in Group III animals. However, degeneration was evident at the apex of the villi.

Group V (Liv.52 only) – Liv.52 therapy in the absence of Cd exhibited hyperactivity of the cells in the intestinal villi causing their enlargement, which in turn resulted in the congestion of the lumen (Fig. 7).

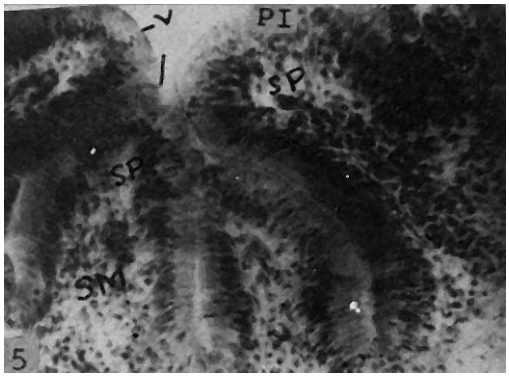

Fig. 5: Section of posterior intestine of Cd-treated (Group II) fish indicating shortening of the villi and necrosis at their tips (x 200).

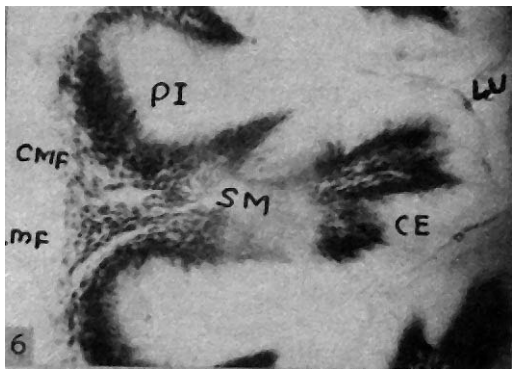

Fig. 6: Section of posterior intestine of Group III (Cd + Liv.52) fish showing hyperactivity of villi cells. No pathological symptoms visible (x 200).

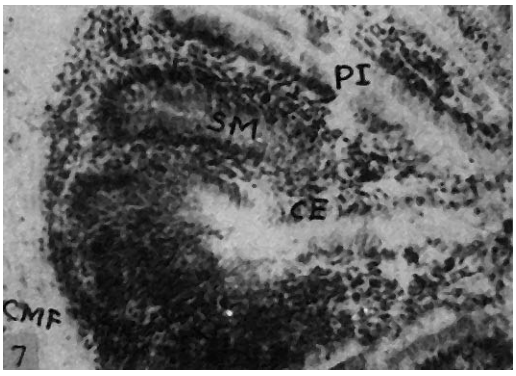

Fig. 7: Section of posterior intestine of Group V (Liv.52 only) fish showing elongated and tapering villi with reduced lumen (x 200).

Key to abbreviations appearing in Figs. 1-7

AI – Anterior intestine
BM – Basement membrane
CE – Columnar epithelium
CMF – Circular muscle fibres
FTV – Flattened tip of villi
DBM – Degenerating basement membrane
GC – Goblet cell
LMF – Longitudinal muscle fibres
LU – Lumen
MF – Muscle fibres
SM – Submucosa
SP – Space
PI – Posterior intestine
V – Villi

DISCUSSION

Eisler (1971) pointed out that salinity reduces Cd toxicity, whereas Carroll *et al.,* (1979) and Calamari *et al.,* (1980) concluded that hardness of water plays an important role in determining Cd toxicity in fish. During the present investigation hardness, alkalinity and chloride contents were found to increase the toxicity of the Cd-contaminated water (Groups II, III and IV).

Enhanced locomotor activity in *Lepomis macrochirus* (Ellegard *et al.,* 1978) and passiveness, poor appetite and unco-ordinated swimming movements in zebra fish *Brachydanio rerio* (Karlson-Norrgren *et al.,* 1985) have been reported due to Cd poisoning. During this investigation behavioural responses of *M. tengara* to Cd were quite similar to those found in zebra fish. It is already known that Liv.52 increases the appetite in mammals. A similar effect of the drug was seen in fish during the present investigation.

Structural damage due to Cd toxicity in fish testes (Sangalang and Freeman, 1974) and gills (Voyer, 1975; Karlson-Norrgren, 1985; Kothari and Saxena, 1988) has been reported. Retarded bone growth due to Cd poisoning has been shown in *Trilobodon fry* (Nakamura, 1976). During the present investigation, necrosis leading to cell death in the columnar epithelial cells at the tips of the villi in the anterior intestine, and necrosis and shortening of villi in the posterior intestine, were evidently seen in *M. tengara* due to Cd toxicity. More or less similar histological changes in fish intestine due to lead (Sastri and Gupta, 1978) and Cd (Stromberg *et al.,* 1983) poisoning have been reported.

Investigations conducted in the past revealed that Liv.52 provided protection against a wide variety of poisons to mammalian liver (Prasad, 1976, 1980), adrenals (Majumdar and Kulkarni, 1977) and intestinal villi in human jejunum (Tripathi *et al.,* 1977). Findings in fish *M. tengara* also indicated that degenerative changes were less when Cd was mixed with Liv.52 (Group III) and in post Liv.52 therapy (Group IV) animals. An increase in the height of the intestinal villi in *M. tengara* increased its absorptive surface area, which ultimately resulted in more efficient food utilization. This may also be confirmed from the increased appetite in Group V (Liv.52 only) fish. An increase in food consumption and more efficient food utilization in laboratory animals due to Liv.52 is already known (Srinivasan and Balwani, 1968).

This study indicates that Cd poisoning brings about changes in the water quality and fish behaviour, and also decreases the food consumption by fish. Besides, Cd also causes structural damage in the fish intestine. It is also demonstrated that Liv.52 provided protection against the degenerative action of Cd and increased the absorptive surface resulting in better food utilization in *M. tengara.*

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