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Efficacy Of Herbal Drug Against The Histological Damage Due To Mercury In The Intestine Of Freshwater Teleost *Heteropneustus fossilis* (Bloch)

KEYWORDS: Intestine, Mercury, Liv_{52}, Recovery

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ABSTRACT *An experimental study was carried out to assess prophylactic and curative effect of a herbal compound (Liv_{52}, manufactured by The Himalaya Drug Co., Mumbai) against mercuric chloride (0.1mg/l) uptake and resulting histopathological alterations in the Intestine of a freshwater catfish Heteropneustes fossilis (Bloch). Histochemical results indicated that while, heavy accumulation of metal was seen in Intestinal wall in Hg exposed fish, only traces of metal were seen in Hg+ Liv_{52} treated group. In normal recovery higher Hg concentration was seen in serosa and columnar epithelium whereas, recovery with Liv_{52}revealed only traces of metal in the basement membrane. Similarly, structural damage in Intestinal tissue was less in Liv_{52} fed fishes both, in the Hg+Liv_{52} treated group as well as, in recovery with the drug. This suggests prophylactic and curative effects of herbal compound against mercury toxicity in the catfish Intestine.*

Introduction

The threat of pollution to the aquatic life is caused by heavy metals, industrial pollutants, domestic, agricultural wastes, etc. Kalay and Canli (2000); Beaumont *et. al.* (2000), Almeida *et. al.* (2001), Reddy *et. al.* (2011), Opaluwa *et. al.* (2012), Haloi *et. al.* (2013) and Karthigayani *et. al.* (2014) also found in their studies that the pollution is exerting stress on the aquatic life.

Khaled (2004) and Ali and Fishar (2005) reported concentrations of metal accumulation in the fish organs. Mercury is highly toxic non-essential heavy metal and has a unique property of accumulation and reaches human tissues through food chain. Devlin (2006) reported Mercury among various environmental pollutants deserve special attention due to its markedly increased use in industry and agriculture.

Intestine is important for absorption of nutrients. Miller (1981) observed it to be very sensitive to Hg exposure. In past, few reports had appeared on histochemical distribution of Hg in fish organs. Kumar and Kothari (1990), Bhoraskar and Kothari (1993) reported such findings. Elizabeth *et. al.* (1992) and Gupta and Kumar (2006) investigated few species of fishes for histopathological changes in vital organs exposed to Hg Salts.

Kothari *et. al.* (1990) documented preventive and curative effects of an indigenous drug Liv_{52} for heavy metal toxicity in fish. Rathore and Verma (1988), reported similar effects on mammalian organs.

In view of the protective action of herbal drug against Hg accumulation and Hg induced tissue damage in Intestine of fresh water teleost *H. fossilis*, this study hadbeen undertaken. The role of drug, if any in the recovery process in the Hg exposed fish had also been undertaken.

Material and Methods

H. fossilis procured from fish farm were acclimatized to laboratory conditions in glass aquaria for seven days. Stock solution of $HgCl_2$ was prepared as per guidelines of APHA (1975). Liv_{52} is an indigenous herbal compound and is known hepatoprotective drug. Composition of the drug was mentioned in the earlier study, conducted by Rathore and Verma (1988). The experimental concentration of $HgCl_2$ was 0.1 mg/l. Acclimatized fish were divided into groups of 25 each as under.

The food and drug were fed at the rate of 30 and 10 mg/day/fish, with few drops of liquid paraffin, to the fishes of all aquaria respectively. On every fourth day water of all aquaria was changed and fresh metals, solution was added to experimental groups. The fishes were divided into various groups as mentioned in the table 1.0.

Fishes from groups I, II and III were sacrificed on 30^{th} days while those of recovery experiment were sacrificed on 60^{th} day and accumulation of Hg in fish Intestine was demonstrated by using Sulphide Silver Method for heavy metals, as described by Pearse (1972). Mercury was localized in tissue section as brownish black deposits of mercury sulphide.

For histopathological studies Intestine was processed in routine procedure and double stained with Haematoxylin and Eosin.

Results and Discussions

In Histochemical studies (Fig. 1.0) no traceable amount of Hg was localized in the Intestine of control group. In group II, moderate Hg distribution was noticed in the muscle layers, sub mucosa and columnar cells with high concentration in serosa (S). Columnar cells and basement membrane (BM) also displayed Hg accumulation. Whereas in group III, only fine granular and scattered particles of metal were observed in the serosa (S), Longitudinal muscles (LM) and Circular muscles (CM) as well. Metal was mainly concentrated in diffused form in the component tissues of the villi (V).

It had been shown that after absorption and distribution in the organism, toxicants are excreted rapidly or slowly. The process of uptake and elimination of Silver reported by Coleman and Cearley (1974) is well known phenomenon in fish.

Earlier investigation carried out by Boudou *et. al.* (1991)

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with inorganic and organic Hg revealed very specific properties of the Intestinal barrier regarding Hg fixation and absorption. The result of this study supports the views of Boudou and Ribeyre, (1983), as low amount of Hg was detected in catfish Intestine with the intestinal wall and mucosa being the main sites of metal accumulation.

In natural recovery (Group IV A) after decontamination phase, loss of Hg was seen from the villi (V), where metal was retained mainly in the columnar nuclei. As a consequence of redistribution during recovery, circular muscles (CM) also exhibited Hg accumulation. In Drug recovery (Group IV B) Hg was only localised along the shrunken basement membranes (BM) of the villi (V), suggesting faster removal of Hg under the influence of the drug.

Since no increase in Hg concentration during decontamination was noticed. It was concluded that there was no redistribution of Hg and the observed decrease was presumably due to excretion or elimination of Hg from Intestine. This conclusion was consistent with the earlier reports of Boudou and Ribeyre (1983) and Boudou *et. al*. (1991).

The observed difference in the amount of Hg was suggested of the effective role of Liv_{52} in the detoxification process in the Intestine. The results suggested that in relation to Hg deposition, simultaneous treatment of the drug is more effective than post therapy group.

In Histopathological studies (Fig. 2.0), the Intestine of control group exhibited normal histological architecture. In Group II tips of villi were broad and rounded (RV), instead of long and tapering villi (V), as seen in control group. This resulted in tissue free gap (TFG) in lamina propria (LP) and shortening of intestinal villi. The basement membrane and columnar epithelium were found eroded at places and lamina propria was found shrunken.

The structural damages seen in group II, were consistent with the earlier findings reported in fish Intestine due to heavy metal poisoning by Miller *et al.*, (1981), Kothari *et al*. (1990), Saxena and Kothari (1993).

In Group III, Hg induced histological changes were reduced in the presence of the drug Liv_{52}. Changes such as broadening of villus tip and formation of tissue free gap (TFG) were not as prominent as observed in Group II animals.

The studies conducted on the protective action of Liv_{52} in mouse spleen, duodenum and small Intestine by Rathore and Rawat, (1989), were well documented and supported the findings of the present study.

In Natural recovery (IVA) noticeable improvement in histological architecture of Intestine was noticed after the removal of Hg stress. Both mucosa and basement membranes (BM) were complete and smooth as against the Hg treated group. Villi (V) were regaining long and tapering shape. Tissue free gap in lamina propria was very much reduced, indicating recovery of the structural damage. Recovery in the presence of drug (IV B) was more effective as comparison to natural recovery. Height and shape of the villi returned to normal condition. No tissue free gap was seen in the laming propria region and definite improvement in Intestinal architecture was evidently seen.

These results indicated a visible correlation between loss/ elimination of Hg and the structural recovery in fish Intestine after decontamination period.

The observed difference in the site of accumulation of the metal may be attributed to the changes in Hg concentration in ambient water and the prolonged duration of exposure.

Reduction in Hg accumulation in both the recovery groups may be attributed to the redistribution and / or excretion of the metal by the fish as reported by Coleman and Cearley (1974) and Cearley and Coleman (1974).

Conclusion

The findings of this investigation revealed that the sites of active metal deposition and the sites of structural damage were almost same, suggesting a visible correlation between metal accumulation and tissue damage.

In the presence of drug Liv_{52} tissue accumulation of Hg was suppressed which may be due to the effect of Liv_{52} on the binding capacity of metal particles to -SH group of protein and / or by affecting the uptake and elimination of metal by the fish organs. However, further detail studies are needed to understand the mode of protective action of the drug against toxic action of pollutants.

1	Group I	Control fed on normal food
2	Group II	Treated with $HgCl_2$ (0.1 mg /l) and fed on normal food
3	Group III	Treated with $HgCl_2$ (0.1 mg/l) and fed on food containing drug (Hg + drug)
4	Group IV	First 30 days treated with $HgCl_2$ (o.1 mg/l) and fed on normal food then divided into two groups 30 days kept in Hg free water. i.e. IV A Natural recovery 30 days kept in Hg free water and treated with Drug. i.e. IV B Drug recovery

Table 1.0 Experimental Plan

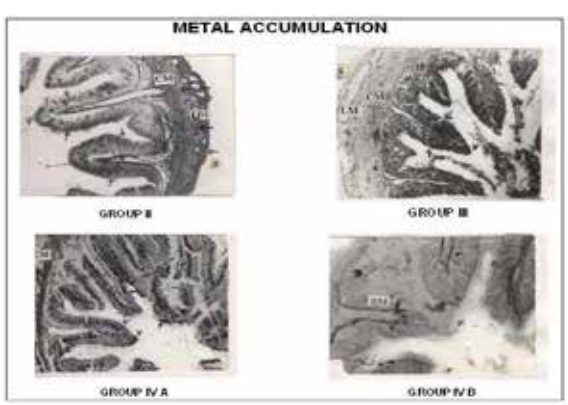

Fig. 1.0 Metal accumulation in the Intestine of various groups

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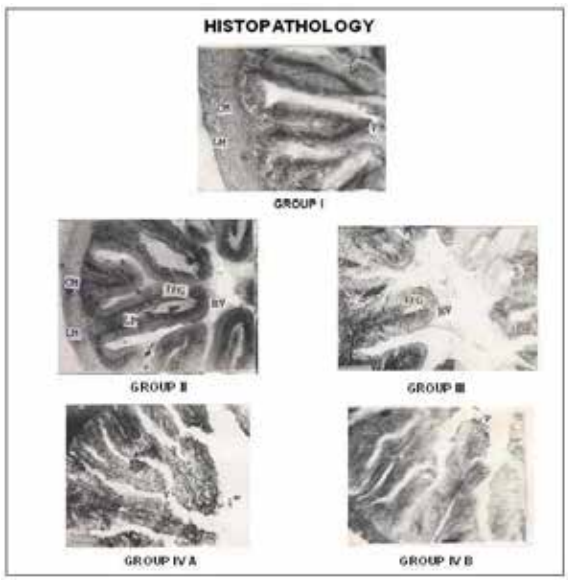

Fig. 2.0 Histopathology of Intestine of various groups

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