Research Article

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HERBAL PROTECTION AGAINST MERCURY UPTAKE AND HISTOLOGICAL DAMAGE IN GILL OF FRESH WATER TELEOST *HETEROPNEUSTES FOSSILIS* (BLOCH)

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Abstract: The protective effects of a herbal compound Liv_{52} were studied against mercuric chloride (0.1 mg/l) induced histochemical and histological changes in gill of a fresh water catfish *Heteropneustes fossils* (Bloch). Heavy metal accumulation was seen in the secondary gill filaments in mercury exposed fish, while moderate Hg deposition was seen in the Hg + Liv_{52} treated group. The effective recovery was seen in the Liv_{52} fed fishes as against the normal recovery. Similarly, the structural damages such as degenerated lamellar epithelium, curved and exposed pillar cells in gill was lesser in the Hg + Liv_{52}, as well as in the recovery with drug. This suggests preventive and curative effect of herbal compound against mercury intoxication in gill of *H. fossilis*.

Keywords: Histological, Histochemical, Gill, Mercury, Liv_{52}, Recovery

INTRODUCTION

Mercury is highly toxic non-essential heavy metal and has a unique property of accumulation over a period of time and reaches human tissues through food chain[1]. Among environmental pollutants mercury deserves special attention due to its markedly increased use in industry[2] and agriculture. Mercury entering in any form in the aquatic environment may be converted to the highly toxic methyl mercury[3] and fish being an important link of the food chain affects them both directly as well as indirectly. It cannot be removed and is rapidly transformed by microorganisms into organic compounds that tend to bio accumulate and biomagnify in animals[4]. The fishes are highly susceptible even to a very low concentration of Hg in the water[5-7]. Due to its markedly increased use in industry and agriculture mercury deserves special attention.

Gill is an important organ for respiration and has been proved to be very sensitive to metals[8-11] and Hg exposure[12]. The entry of Hg from medium is large through the gills[13]. It has been reported that the gill is more or less permeable structure for its absorption[14]. Gill provides one of the major passages for entering the pollutants into other major organs like liver, kidney[15], etc. In recent past few reports have appeared on histochemical distribution of Hg in fish organs[16-18]. A few species of fish have been investigated in the past for histological changes in vital organs exposed to Hg Salts[19-22].

Histological investigations have been considered as reliable biomarkers of stress in fish[23]. These changes are being widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, either in the laboratory or in the studies of the natural water resources. The major great advantage of such histological biomarkers to monitor environment is that it helps to examine the specific target organ. The organs may be gills, kidney and liver, which are responsible for vital functions, like respiration, excretion, accumulation and biotransformation of xenobiotics in the fish[24].

Preventive and Curative effects of an indigenous drug Liv_{52} for heavy metal toxicity is well documented in fish[25] and mammalian organs[26]. In view of the protective action of herbal drug against Hg accumulation and Hg induced tissue damages in gill of fresh water teleost *H. fossilis*, this study had been undertaken. The role of drug, if any in the recovery process in the Hg exposed fish had also been undertaken.

Objective of Research:

The present study was aimed to understand

a. The sites of Hg accumulation in gill of fresh water teleost fish *H. fossilis* (Bloch.).

b. The effect of drug Liv_{52} in reducing the Hg burden from fish gill.

c. The histopathological changes due to Hg toxicity in the fish gill.

d. The effectiveness of drug Liv_{52} in the structural improvement of gill damage due to mercury toxicity.

e. The role of drug Liv52 after Hg toxication, i.e., recovery (decontamination) phase.

f. To observe the correlation between metal accumulation and structural damage, if any.

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MATERIALS AND METHODS

Heteropneustes fossilis (Bloch) is a common Indian catfish known as 'Singhi'. It is widely distributed fresh water catfish which forms an important source of fish food. *H. fossilis* is an air breathing fresh water catfish with a sac like accessory respiratory organ. It is a very hardy fish and can conveniently be maintained in aquarium with a little quantity of water. Due to its hardy nature, easy availability and convenient maintenance under laboratory conditions, this fish was chosen for the present investigation.

The Liv_{52} is an indigenous well known hepato-protective herbal drug. It is manufactured by the Himalaya Drug Company, Mumbai (India). The Composition of this drug was studied earlier also[26].

Stock solution of $Hgcl_2$ (B.D.H.) was prepared in double distilled water. The experimental concentration of $HgCl_2$ was 0.1 mg/l.

The fresh water catfishes *H. fossilis* were collected from the local water body in Ujjain (MP, India) for the experiment purposes. The fishes of average weight 65± 3 gm were selected for the experiment.

These fishes were acclimatized to laboratory conditions in glass aquaria for seven days. Acclimatized fishes were divided into groups of 25 each as under:

Table 1.0 Experimental Plan

Sr. No.	GROUPS	TREATMENT	
1	CONTROL	Without Poison + Plain food	
2	II	Exposed to 0.1 mg/l $HgCl_2$ + Plain food	
3	III	Exposed to 0.1 mg/l $HgCl_2$ + Food containing drug Liv_{52}	
4	IV	First exposed to 0.1 mg/l $HgCl_2$+Plain food for 30 days and then divided in to two groups for recovery studies	
		Group IV 'A'	**Group IV 'B'**
		Maintained in Hg free water for 30 days and fed on plain food (Natural Recovery)	Maintained in Hg free water for 30 days and fed on food containing drug (Recovery with drug)

Fish in all aquaria were fed daily on dried and chopped prawns at the rate of 30 mg / fish / day, while the drug at the rate of 10 mg / kg, body wt / fish / day. Dried and chopped prawns mixed with few drops of liquid paraffin were fed to the animals of all the three groups. Liv_{52} in required amount was mixed with the food of group III and group IV B animals. Food was given daily to all the animals of all the groups. On every fourth day water of all aquaria was changed and fresh metal solution was added to experimental groups.

Five fishes each from groups I, II and III were sacrificed on 30th day; while those of group IV were sacrificed on 60th day and gill was separated.

Histochemical Localization of Mercury

Accumulation of Hg in fish gill was demonstrated by using Silver sulphide method for heavy metals[27]. Hg was localized in tissue section as brownish black deposits of Mercury sulphide. No counter stain was used.

Histopathology of Gill

For histopathological studies gill was fixed in Alcoholic Bouin's solution and processed with a routine procedure to obtain 5µ thick paraffin sections. Sections double stained with haematoxylin and eosins were used for histopathological study.

RESULTS AND DISCUSSIONS

Metal Accumulation (Fig. 1a to Fig. 1d):

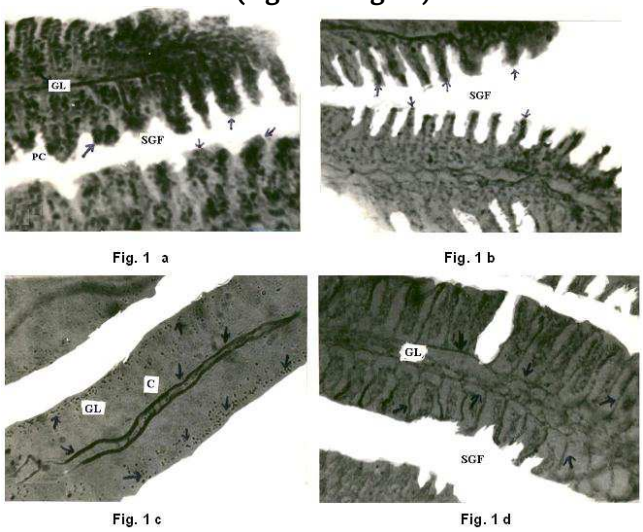

Fig. 1 a: T. S. of Gill exposed to $Hgcl_2$ showing heavy Hg deposition in the secondary gill filaments and Pilaster cells (x150). **Fig. 1 b**: T. S. of gill of Hg + drug treated group exhibiting reduction in Hg concentration in the gill. Metal is localized in the Pilaster cells (x150). **Fig. 1 c**: T. S. of gill of natural recovery (IV A) group showing Hg accumulation along the tips of the secondary gill filaments and in the cartilage (x150). **Fig. 1 d**: T. S. of gill of drug recovery (IV B) showing low concentration of Hg in the pilaster cells and in gill epithelium (x150).

SGF-Secondary Gill Filament; **GL**- Gill Epithelium; **PC**- Pilaster Cells; **C**-Cartilage; Arrow indicates site of Hg deposition.

Group I (Control)

No traceable amount of Hg was localized in the gill of control fish.

Group II ($HgCl_2$ treated)

Secondary gill filaments were heavily loaded with Hg including gill epithelium and pilaster cells

Group III ($HgCl_2$ + Drug)

Moderate deposition of Hg was found in the secondary gill filaments in contrast to heavy deposition noticed in the Group II.

Group IV A (Natural Recovery)

Very fine and scattered metal granules were observed in the gill epithelium particularly at the tip of

secondary gill filaments. The gill cartilage also exhibited Hg deposition.

Group IV B (Drug Recovery)

Very low metal accumulation was localized in blood spaces of the gill filaments with diffused metal deposition in gill epithelium.

Histopathology (Fig. 2a to Fig. 2e):

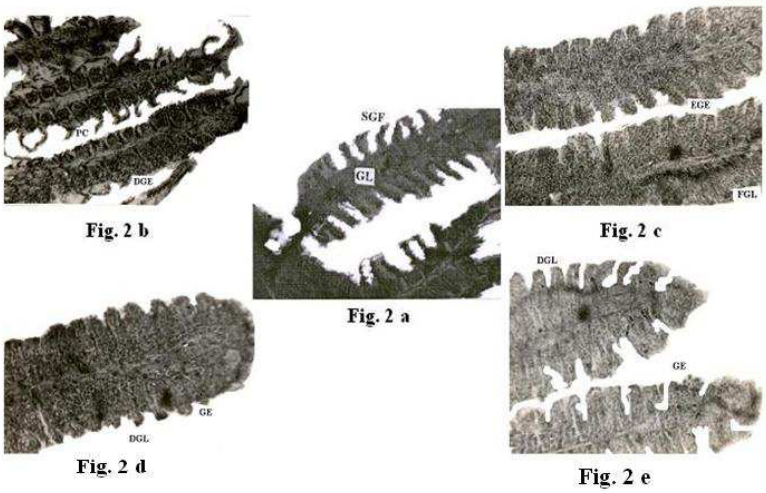

Fig. 2 b Fig. 2 a Fig. 2 c

Fig. 2 d Fig. 2 e

Fig. 2a: T. S. of gill of control fish showing normal structural of gill lamella (x150). **Fig 2. b**: T. S. of gill of $Hgcl_2$ treated fish showing naked pillar cells due to degeneration of gill epithelium (x150). **Fig. 2 c**: T. S. of gill of Hg + drug treated fish exhibiting erosion of gill epithelium. Histology of gill is better than earlier group (x150). **Fig. 2 d**: T. S. of gill after decontamination phase showing recovery of gill epithelium and straight row of pilaster cells (x150). **Fig. 2 e**: T. S. of gill of post therapy group showing better histological architecture of gill filaments with separated tips (x150).

GL- Gill Lamellae **SGF**- Secondary Gill Filament **PC**- Pilaster Cells **DGE**- Degenerated Gill Epithelium **GE**- Gill Epithelium **EGE**- Eroded Gill Epithelium **FGL**- Fused Gill Epithelium **DGL**- Distinct Gill Lamellae

Group I (Control)

Gill of control fish exhibited normal structure. Secondary gill filaments were displaced on the gill lamellae maintaining a definite gap in between the neighboring filaments. Gill epithelium was normal and distinct.

Group II ($HgCl_2$ Treated)

Severe structural damage was noticed due to Hg toxicity in the gill tissue. The gill filaments lost their identity and lamellar epithelium was damaged and degenerated. The supporting pillar cells were seen curved and exposed due to degenerated gill epithelium.

Group III ($HgCl_2$ + Drug)

The gill damage was lesser than the Group II fishes. Unlike Group II the gill epithelium was not degenerated completely but found eroded at places. The tips of gill filaments were free but in some cases it was found fused with neighboring filaments.

Group IV A (Natural Recovery)

Noticeable structural recovery was observed during decontamination phase. Gill epithelium degenerated due to Hg poisoning was regenerated. The gill filaments were still tightly pressed against each other except there tips, which were separated off. The overall gill architecture was observed better than of the Group II.

Group IV B

Better gill recovery was observed in the fish fed on Liv_{52} during decontamination period. Gill filament was almost resumed normal structure. Length wise the proximal half of the filaments was free and distinct. Gill epithelium in the free part of the filament resumed its normal structure.

DISCUSSION

In the gill of group II (Hg alone), Hg was distributed through the component tissues in higher concentrations. It is in accordance with the earlier reports[28-30]. The entry of Hg from medium is large through the gills[31]. Gill is more or less permeable structure for its absorption[32]. It had been shown that the efficiency of Hg absorption through the gill epithelium varies considerably according to the chemical form of the metal added to the water[33, 34].

While in group III (Hg + drug), mercury was restricted only to pilaster cells and tips of the gill filaments. It was presumed that drug interferes with the binding capacity of Hg in fish tissue there by, affecting its accumulation as well as, pattern of distribution in the target organ of catfish. Similarly, the protective role of drug Liv_{52} had been shown in various fish organs[35, 36] against Cd toxicity.

The result of 30 days recovery study revealed a reduction in Hg burden in the gill of catfish after cessation of Hg exposure and maintenance of fish in Hg free water. This noticeable reduction in Hg contents may be either due to the excretion or redistribution of Hg. Since there was no increase in Hg concentration during decontamination phase in the gill, it was concluded that at least there is no recycling/redistribution of Hg and the observed decrease was presumably due to excretion or elimination of Hg from the gill. This conclusion was consistent with the earlier reports[32, 37]. The sites of Hg retention after decontamination were different in the gill tissues. In the natural recovery, while Hg was retained by the tips of the secondary gill filaments, it was found in the gill epithelium in the Liv_{52} treated recovery group.

Gill covers about 60% surface area of the fish and its external location renders it the most vulnerable target organ for aquatic pollution[38]. The gills are the

perfect illustration of donor organs during decontamination phase, the metal being transferred directly to the surrounding medium, but especially via the blood to other tissues[32].

The exposure of *H. fossilis* 0.1 mg/l $HgCl_2$ caused extensive damage in the gills of the catfish. Due to damaged gill epithelium bare columns of pilaster cells were left disturbing the lamellar blood flow[22, 39].

However, when the Hg exposure was accompanied with the drug therapy, only erosion of lamellar epithelium was noticed, suggesting reduction in the Hg toxicity in gills of *H. fossilis*. Earlier report on Cd toxicity in the gills of *Mystus tengara*[36] also suggested protective role of Liv_{52} in tissue and was in accordance with the present finding in *H. fossilis*.

During natural recovery gill filaments though tightly pressed against each other exhibited regeneration of the gill epithelium and pillar cells indicating a returning towards normalcy. In the drug recovery group also the structural improvement in gill was better than observed in normal recovery. The Protective role of drug Liv_{52} was consistent with earlier findings[25, 26]

The results indicated a visible correlation between loss/elimination of Hg and the structural recovery in the fish gill after decontamination period. The improvement in histological architecture may be attributed to the removal of Hg bound to the –SH group of protein, which is evident from the histochemical studies of the Hg distribution in *H. fossilis,* as the Hg burden after recovery phase was reduced in fish gill. Further, faster recovery in drug treated fish gill was indicative of the possible detoxifying action of the drug Liv_{52}.

CONCLUSIONS

On the basis of findings of this experimental study in *H. fossilis*, following conclusions were drawn:

- 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 proteins and/or by affecting the uptake and elimination of metal by the fish organ.
- In general the sites of structural damage and sites of active metal accumulation were almost same. Suggesting a visible correlation between metal accumulation and tissue damage.
- Hg causes severe structural damage to fish tissue. On the contrary, in both pre and post treatment (recovery) with the drug Liv_{52}, structural damage is noticeably reduced.
- These results suggest that Hg is highly toxic to fish and the drug Liv_{52} plays a protective role against Hg induced toxic changes in gill of *H. fossilis*. However, further detail studies are needed to understand the mode of protective action of drug against toxic action of pollutant.

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