Mercury Induced Biochemical Changes in the Intestine of Fish *Cirrhinus mrigala*

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Abstract:

Aquatic animals are significantly affected by oxidative stress caused by environmental pollutants. In last few decades the aquatic animals are exposed to elevated levels of heavy metals continuously. Mercury (Hg) is a wide spread metal pollutant of high toxicity not only to higher vertebrates, but also to aquatic animals like fishes. The methylation of mercury is the key step in the transport of mercury in aquatic food chains. The present study has been undertaken to explore the toxic effects of mercury on intestine of a teleost fish *Cirrhinus mrigala* and to detect the biochemical profile after the exposure. The biochemical changes after mercury exposure are studied by Fourier transform infrared spectroscopy (FTIR). The spectroscopic techniques like FTIR, EDX, Absorbance and PL can be used as a firm tool to detect the impact of toxic elements. The energy dispersive X-ray spectroscopy (EDX) has been used to understand the carbon and oxygen percentage. Optical behavior of tissue has been studied by absorbance and photoluminescence. Field emission scanning electron microscopy (FESEM) is the tool used to study the surface morphology of intestine. The present study gives a manifold confirmation to toxicant impact and can be used to correlate the overall biochemical status of the tissues with histopathological changes undergone at cellular level after chronic exposure to mercury.

Keywords – *C. mrigala*; mercuric chloride; toxicity; spectroscopy

1. Introduction

The heavy metal contamination is getting a growing concern as it affects the terrestrial and aquatic environments and ultimately the human health. Mercury is extremely destructive metal when expelled in environment. The industrial mercury poisoning at Minamata Bay has proved mercury as a unique environmental toxicant. Consequent release of mercury containing wastes into the environment increases the toxicity risk. Mercury is highly toxic to aquatic organisms. The discharge of mercury in resources may result in acute and chronic toxicity in fish. Mercury was consistently most toxic metal in the series of Hg, Cd, Cr^{+6}, Ni and Zn for fresh water annelids, insects, and gastropods (Rehwoldt et al.,1972), crustaceans (Wilson and Connor, 1971), and fish (Weir and Hine,1970). Once mercury enters in the organism it draws various toxic effects. For healthy fish production it is very important to evaluate the harmful effects of heavy metals. (Cunha et al., 2007). Fish are considered sensitive indicators of aquatic pollution and tend to accumulate both organic and inorganic forms of mercury (Gochefeld, 2003). In fact, several studies have reported that some fish species are far more sensitive to heavy metals toxic effects as compared to mammals (Kelley et al., 1998). Among heavy metals, mercury has been selected for the present study as it is a wide spread metal pollutant of high toxicity not only to warm blooded vertebrates, but also to aquatic animals including fishes. (Begum and Sengupta, 2014). In view to the above and there are still little data on mercury exposure and its effects in different organs in tropical fish (Mela et al., 2007), the present study was undertaken to evaluate mercury effect on biochemical profiles of *C. mrigala*.

The intestine is a complex multifunctional organ. In addition as the principal organ of digestion and absorption it works for water and electrolyte balance and endocrine regulation of digestion and metabolism. *C. mrigala* is a popular fresh water fish with strong immune system, quality proteins and is consumed all over the parts of India. The fish forms a famous breed used in aquaculture and available throughout the year, hence fits as an excellent experimental model.

Spectroscopic tools like Fourier Transform Infrared spectroscopy, photoluminescence, are being used extensively to probe quantitative biochemical information in biological samples (Akkas et al.2007) (G. M. Lohar et al., 2014) (G. M. Lohar et al., 2014). FE-SEM provides surface morphological details, compositional analysis of carbon and oxygen is studied by EDS, optical response of biological sample is studied by UV visible spectrophotometer and photoluminescence can be studied by spectrofluorometer. The present paper, explores the mercury induced biochemical changes in the kidney tissues of freshwater fish *C. mrigala*.

2. Materials and methods

2.1 Biological material

The live fresh water teleost *C. mrigala* of average length 18-20 cm and average body weight 70-75g. were collected from a reservoir at Kalambe near Kolhapur, M. S. India. Animals were acclimatized to laboratory conditions for 15 days in glass aquarium containing dechlorinated tap water and continuous aeration. Fish were fed ad libitum and were maintained on a photoperiod with 12h light and 12h dark. Well acclimated and healthy fish were selected for the experimental work. The water was tested for selected physico chemical parameters.

2.2 Exposure

Chemicals-Analytical grade Mercuric chloride ($HgCl_2$) (BDH) was used without further purification. Stock solution of mercuric chloride was prepared by dissolving analytical grade mercuric chloride in double distilled water. The sub lethal concentration selected for chronic toxicity experiment were $1/20^{th}$ of LC_{50} and $1/10^{th}$ of LC_{50} ($1/20^{th}$ and $1/10^{th}$ of LC_{50} values 0.0206 ppm and 0.0402 ppm) concentration of mercuric chloride. The desired concentration of mercuric chloride was prepared from stock solution. The acclimated test animals in a group of 10 were exposed to the sub lethal concentration for a period of 30 days. A control set was run simultaneously. The experiment was arranged in triplicates. The water with toxicant renewed daily and fish were fed ad libitum during the period. The fish were sacrificed after 30 days and the desired tissue was pulled out. Sample preparation -The intestine tissue was blotted and dried for 72hrs in oven at 60^0C.and then ground in mortar and pestle to obtain intestine powder. The powder was used as a sample for further analysis.

The vibrational analysis of intestine of *C.mrigala* have been studied using the perkin elmer, USA, Fourier transform infrared spectroscope (FTIR). The surface morphology has been studied using the Mira 3, Tescan, chez republic, field emission scanning electron microscope. Energy dispersive spectroscopy has studied using the Mira 3 Tescan and oxford instrument, United Kingdom. Absorption spectra were recorded at room temperature and near to normal incidence using a UV-1800 Shimadzu, Japan. Photoluminescence has been studied using the fluoromax-4, horiba instrument PVT, Japan.

3. Results and discussion

3.1Fourier Transform Infrared Spectroscopic studies (FTIR)

The present study is carried out to analyze the toxic effects of mercury in the intestine tissues of fresh water fish *C.mrigala* by using FTIR spectroscopy. The FTIR shows the transmittance peak at 3378, 3429 and 3437 cm^{-1} for control, and samples exposed to sub lethal concentration of mercuric chloride 0.0206 and 0.0402 ppm ($1/20^{th}$ of LC_{50} and $1/10^{th}$ of LC_{50}), respectively. All these peaks are N-H stretch bond and 1˚, 2˚ amines, amides functional groups. These peaks are due to the protein content present in intestine of fish *C.mrigala*.

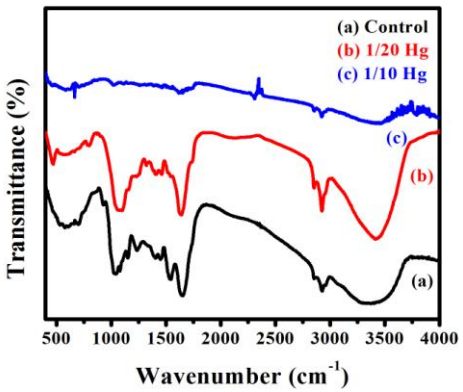

Fig.1 FTIR spectra of control and mercury exposed *C-mrigala* Intestine (a) control, (b) 1/20th $HgCl_2$, (c) 1/10th $HgCl_2$

The peak at 2929 and 2852 cm^{-1} for all samples represents the C-H stretch from alkanes of lipids. The decrease area of the transmittance for 2929 and 2852 cm^{-1} is due to functional groups of lipids. The peak at 2310 cm^{-1} for 0.0402 ppm of mercuric chloride is C≡N stretch of nitriles for proteins. This peak is absent at control and the tissues exposed to 0.0206 ppm concentration of mercuric chloride. The arising of this peak at higher concentration is indicating the toxic effect of mercury. The peak at 1650, 1630 and 1641 cm^{-1} for control, and samples exposed to sub lethal concentration of mercuric chloride 0.0206 and 0.0402 ppm respectively indicate -C=C- stretch for alkanes of proteins. The peak at 1539 cm^{-1} for control sample belongs to N-O asymmetric stretch for nitro compounds. This peak is absent for mercury exposed samples, due to mercuric exposure, it indicate the mercury exposure is able to change the vibrational spectrum of intestine of fish of *C.mrigala.* The peak at 1037 cm-1 for all three samples for C-N stretch for aliphatic amines of polysaccharides.

Table.1 General band assignment of the FTIR spectra of control and mercury exposed C-mrigala intestine

Sr. No.	Control	1/20	1/10	Bonds	Functional group
1	3378	3429	3437	N-H stretch	1˚, 2˚ amines, amides
2	2929	2929	2929	O-H stretch C-H stretch	carboxylic acids, Alkanes
3	2852	2852	2852	C-H stretch	Alkanes
4	--	--	2310	C≡N stretch	Nitriles
5	1650	1630	1641	-C=C– stretch	Alkenes
6	1539	---	---	N-O asymmetric stretch	Nitro compounds
7	1037	1037	1037	C-N stretch	Aliphatic amines

The FTIR spectra thus provide the intestinal biochemical profile of control and fish exposed to sub lethal concentration of mercuric chloride. The decrease in band areas and absorbance intensity indicate destructive effect of mercury.Samuel et al., 2005 reported that arsenic treatment decreases the protein content in the brain tissues of rat. Palanippan and Renju (2009) reported significant alterations in the protein after zinc exposure in *Labeo rohita*. The FTIR study on liver of a fresh water fish *Oreochromis mossambicus* were reported by Venkataramana et al., 2010. Ammonia induced biochemical changes in

muscle tissue of *Cyprinus carpio* were reported by Senthamilselvan and Chezhian (2014). Jagadeesan et al, (1999) evaluated that the depletion in protein profile brings diversification of energy to meet the impending energy demands during the toxic stress. A similar observation in *Cyprinus carpio* (Jana and Bandyopadhya, 1987) and *Catla catla* (Vincent and Ambrose, 1994) were reported after exposure to lindane and cadmium respectively. In the present study a considerable decrease in different functional groups of lipids, proteins and carbohydrates in intestine of mercury exposed fish indicate alterations in major biochemical components further suggesting functional deformities in intestine.

3.2 Field emission scanning electron microscopic study (FSEM)

Sublethal concentration of mercuric chloride has the capacity to bioaccumulate thereby altering the normal functional activities (Begam,2014). The present study was conducted to determine the toxicity of mercury at cellular levels in *C. mrigala*. The surface morphological study has been carried out for intestine of control and mercury exposed fish. The Fig. 2 (A1 and A2). Fig. 2 (B1 and B2) show degeneration of microvilli, damaged mucosal folds, distorted mucous cells. Vacuolation is common in all areas of tissue. The Fig.2 (C1 and C2) shows extensive deterioration of brush border of intestinal villi. The cell population is observed to be decreased after mercury exposure. In the intestine, final digestion and absorption takes place. Villi are the absorptive area of digested food. FESEM study revealed loss of microvilli, disruption of primary and mucosal folds, severe necrosis and cracked clay appearance of intestinal tissue. Altered structure of intestinal villi lead to functional impairment and disturb the fundamental function of absorption. The absorption of inorganic mercury in intestine of rainbow trout *Onchorhynchus mykiss* was studied by Hoyle and Handy (2005). Haque et al., (2012) reported Fluoride toxicity in the intestine of *Channa punctatus*, with disruption of primary and secondary mucosal folds.

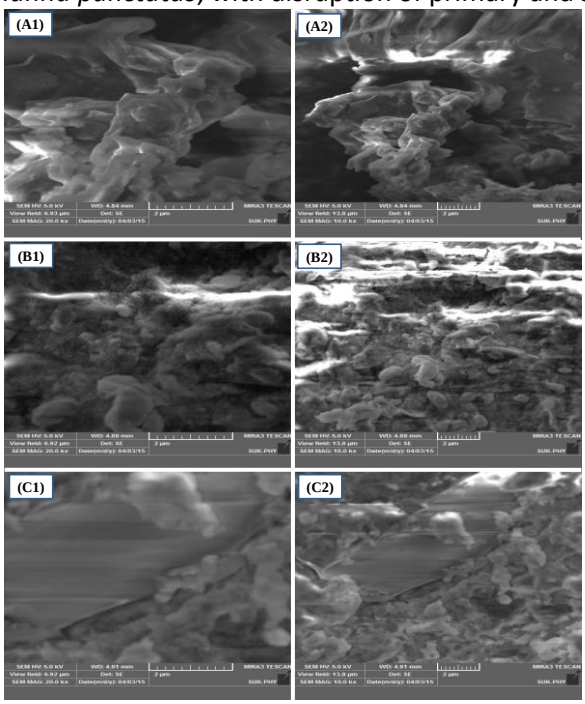

Fig. 2 FE-SEM images of control and mercury exposed *C-mrigala* Intestine (A1) Control X=50kx, (A2) Control X=25kx, (B1) 1/20th $HgCl_2$ X=50kx, (B2) 1/20th $HgCl_2$ X=25kx, (C1) 1/10th $HgCl_2$ X=50kx, (C2) 1/10th $HgCl_2$ X=25kx.

3.3 Energy dispersive X-ray spectroscopic study (EDX)

SEM together with EDX is used in toxicology and pathology for determining exogenic and endogenic toxic substances. (Kopani, 2007; Jurdak, 2008). EDX analysis of tissue confirms the presence of toxic material. EDX enables to detect the materials in animal tissues which do not occur in normal circumstances. The use of EDX analysis is a suitable apparatus for studying the subtle material penetrating the animal tissues and organs. The EDX analysis has been carried out to reveal the effect of mercury on carbon oxygen percentage of intestine tissue. The EDX spectrum reveled carbon and oxygen. For the control sample the weight and atomic percentage of carbon is 31.85% and 38.36%, respectively. While, that for oxygen is 68.15% and 61.14%, respectively this is shown in fig.3 (a). The weight and atomic percentage of carbon for tissue samples with a chronic exposure to sub lethal concentration of 0.0206 ppm is 80.65% and 84.73%, respectively, while, that for oxygen is 19.35% and 15.27%, respectively this is shown in fig.3 (b). At the 0.0402 ppm concentration mercuric chloride chronic exposure the weight and atomic percentage of carbon is 81.22% and 85.21%, respectively. Besides, oxygen weight and atomic percentage is 18.78% and 14.79%, respectively this is shown in fig. 3(c). The first chemical element with the highest percent in animal tissue is oxygen. In the present study, the increase in atomic and weight percentage of carbon is significant and is due to toxicant exposure. The gradual decrease in weight and atomic percentage of oxygen is due to excessive stress of toxicant which might have reduced oxygen in intestine of *C. mrigala*. As little amount of $HgCl_2$ has been used for the exposure, hence this is not observed in EDX of kidney.

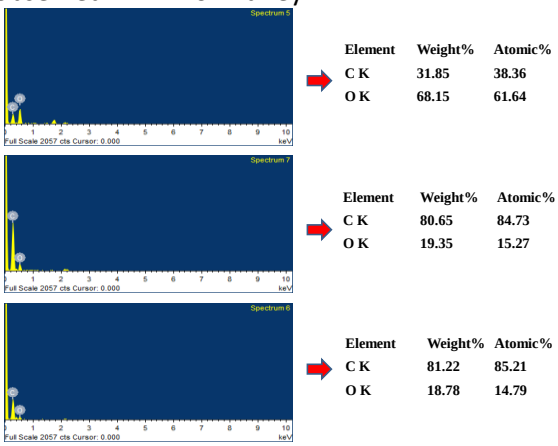

Fig. 3 EDX spectra of control and mercury exposed *C-mrigala* Intestine (a) control, (b) $1/20^{th}$ $HgCl_2$, (c) $1/10^{th}$ $HgCl_2$

3.4 Optical absorbance

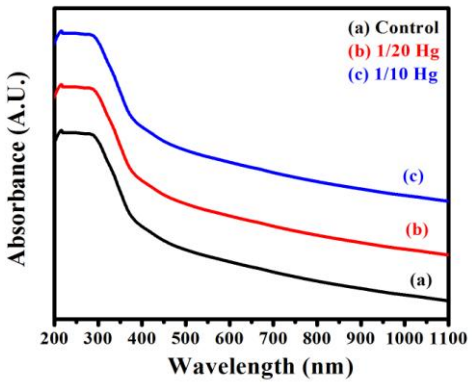

Fig. 4 Optical absorbance spectra of control and mercury exposed *C-mrigala* Intestine (a) control,(b) $1/20^{th}$ $HgCl_2$, (c) $1/10^{th}$ $HgCl_2$

Optical behavior of intestine of *C. mrigala* has been studied using UV- vis spectrophotometer (Lohar et al 2014). The optical absorbance has been studied by dissolving the prepared intestine powder in methanol. The optical absorbance has been observed near at 300 nm this is shown in fig.4. It means that intestine of *C. mrigala* respond near to UV-light. The slight increase in absorbance in visible region from higher to lower wavelength region has been observed. The observed absorbance peak at 300 nm is near UV region. After the mercuric exposure change has been observed in absorbance intensity. The change in absorbance intensity is also indicating the effect of $HgCl_2$ exposure.

3.5 Photoluminescence

It is fascinating to observe that biological systems continuously emitting weak light (Cifra et al., 2014, Pospisil et al., 2014). Spectrofluorometer is tool which helps us to understand the emission of biological tissue. Photoluminescence study has been carried out with the help of spectrofluorometer. The emission of *C. mrigala* has been studied with external excitation of 300 nm with help of spectrofluorometer. The emissions have been observed at 396 nm for intestine of *C. mrigala* this is shown in Fig. 5.

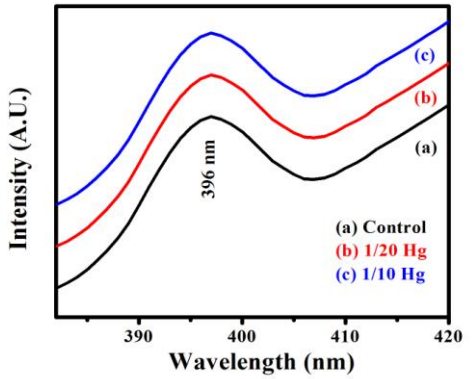

Fig. 5 Photoluminescence spectra of control and mercury exposed *C-mrigala* Intestine (a) control, (b) $1/20^{th}$ $HgCl_2$, (c) $1/10^{th}$ $HgCl_2$

4. Conclusions

In the present paper changes in biochemistry of fish intestine after mercuric chloride exposure has been discussed. The results of FTIR indicate that intestine is a complex of many organic compounds. The surface morphology shows the alterations due to mercuric exposure, which confirms functional alterations in intestine. The sharp absorbance has been observed at 262 nm for all samples. The intestine of *C.mrigala* shows the strong emission at 378 and 440 nm but mercuric exposure is responsible for change in intensity only. The mercury intoxication induced alterations in intestine as significant difference in absorbance intensities reflect the alterations in major biochemical components. The biochemical changes in intestine reduce the food quality of fish. All results are the index of stress in *C. mrigala* after mercury exposure.

5. References

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