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Research Article

CADMIUM CHLORIDE INDUCED CHANGES IN PROTEIN MOLECULES OF THE FRESHWATER FISH *CIRRHINUS MRIGALA* (HAMILTON)

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ABSTRACT

The fresh water fish Cirrhinus mrigala (Hamilton) was exposed to the heavy metal Cadmium chloride for 24, 48, 72, and 96 h, and the consequential LC50 values were calculated using Finney's probit analysis. The LC50 values obtained for 24, 48, 72, and 96 h were 317.5, 316.5, 316.0 and 315.5 respectively. Later the fish were exposed to 96 h acute lethal and sub-lethal concentrations and the changes in protein subunits were analyzed in the tissue of the vital organs such as brain, liver, muscle, gill and kidney using SDS-PAGE electrophoresis. The results revealed that among the protein molecules some became faded when compared to control fish protein molecules, whereas some protein bands disappeared. The analysis was made with the help of standard protein marker. The changes are more pronounced in the tissue of liver and muscle, which may be due to the involvement of liver in the detoxification mechanism. Whereas in case of muscle the changes in the protein banding pattern may be due to the consumption of energy through erratic movement caused due to the toxicant stress. It was also observed that the changes in kidney protein molecules is also more and this may be due to the accumulation of cadmium chloride in kidney tissue. The results obtained were discussed at length with the available literature.

Keywords: : Cadmium chloride, *Cirrhinus mrigala,* Protein molecules, Liver, LC_{50} and SDS-PAGE.

INTRODUCTION

Among all types of pollution, aquatic pollution is of greater concern as each and every kind of the life depends on water. Among all types of aquatic pollutants, heavy metals are of greatest concern. Heavy metals when reach the aquatic bodies deteriorate the life sustaining quality of water and cause damages to both flora and fauna (Nriagu and Sprague, 1987; Nriagu, 1996; Mason, 1996; Kotsanis and Georgudaki, 1999; Zyadah and Abdel-Bakey, 2000; Lliopoulou-Georguadaki and Kotsanis,n 2001; Verma et al., 2005; Samanta et al., 2005; Sharma and Agarwal, 2005). Cadmium is discharged in large quantities from battery and inverter manufacturers, dyeing, printing and electroplating units. It tends to accumulate in tissues of biotic, flora- fauna and has deleterious effect on fish (Barman and Lal, 1994).

The development of electrophoretic techniques makes it possible to detect the protein composition. Multiple factors including season, physical and chemical properties of water can play a significant role in metal accumulation in different fish tissues (Hayat et al., 2007; Romeo et al., 1999). Several studies (Ademoroti, 1996; Heath, 1987; Allen, 1995; Karthikeyan et al., 2007) have also revealed that fish are able to accumulate and retain heavy metals from their environment depending upon exposure concentration and duration as well as salinity, temperature, hardness and metabolism of the animals. These are termed as 'conformational forms (Lumry and Erying, 1954).

The potential value of electrophoresis in this study is based on the hypothesis that stress conditions may cause significant qualitative and quantitative changes in the proteins of different tissue exposed to the toxicant. Such changes might reflect an altered antibody synthesis, protein biosynthesis, cellular leakage or perhaps other events resulting directly or indirectly from the stress. The fish, as a bio-indicator species, plays an important role in the monitoring of water pollution because it responds with great sensitivity to changes in the aquatic environment. The sudden death of fish indicates heavy pollution; the effects of exposure to sub-lethal levels of pollutants can be measured in terms of biochemical, physiological or histological responses of the fish organism (Veeraiah, 2001); Indian major carp fish Cirrhinus mrigala (Hamilton) is one of the most common freshwater fish used in toxicological studies, because it represent a number of characteristics that may make it an appropriate model that can be used as indicator species in bio-monitoring programs.

This unique combination of protein biomarkers could constitute a sensitive tool for early detection of the disease. However, no doubt that gel electrophoresis using sodium dodecyl sulfate (SDS) allowing of proteins molecular weight-based separations, has been widely used (Nakatogawa. H, 2012; Moroni A V, 2010). But for the identification this technique requires extensive data to cover all known target species (Leisner et al. 1994).

In the present study an attempt has been made to determine the molecular weights of the individual subunits of protein by SDS-PAGE from the tissue of vital organs such as gill, brain, liver, muscle and kindney of fish Cirrhinus mrigala after it had been exposed to Cadmium chloride for 96 h.

MATERIALS AND METHODS

Reagents

The protein molecular mass markers for SDS-PAGE experiments were obtained from Rankem. Biochemical grade urea, SDS, β-mercaptoethanol, were purchased (Rankem laboratories), highly pure water was obtained from Milli-Q water system (Millipore, Bradford, MA, USA).

Apparatus

The experiments were carried out on Mini-PROTEAN 3 Cell electrophoretic apparatus (Bio-Rad,Hercules, CA, USA) and P/ACE MDQ capillary electrophoresis system (Beckman Instruments, Fullerton, CA, USA), equipped with an UV detection system. The Polytron homogenization apparatus (PT) 2100 from Kinematica (Littau-Lucerne, Switzerland) for homogenization of fish Cirrhinus mrigala (Hamilton) tissue samples was also used.

Sample preparation

The fish organs of gill, brain, liver, muscle, and kidney were homogenated in 10% Trichloroacetic acid and 1%of the homogenized was centrifuged at 8000 rpm for 10 min in cooling centrifuge. The pellet was washed twice with ice cooled acetone, again centrifuged at 8000 rpm for 10 minutes. The pellet was dissolved in sample buffer (0.5M Tris-HCl, pH 6.8-2ml, 40% glycerol -1.6 ml, 10% SDS-3.2 ml, α-mercaptoethanol- 0.8 ml, 0.1% (W/V) bromophenol blue-0.4ml) and boiled in water bath at 950C for 10 minutes.

Preparation of Gel slab

The glass plate's sandwich was assembled using two clean glass plates and 1mm Teflon spacers. The glass plates were sealed with 1% agar solution. Resolving gel solution 12.5 % (1.5 M Tris- HCl, PH 8.8 -2 ml, 30 % Acrylamide-3.2 ml, 10 % SDS-0.5 ml double distilled water-1.8 ml, TEMED-0.015 ml, Ammonium per sulfate-0.5 ml) was prepared and poured in between the clamped glass plates. To avoid entrapment of any air bubbles, the gel solution was overlaid with distilled water. The plates were left undisturbed for 30 min for polymerization of the gel. After gel polymerization, overlaid water was removed and rinsed with stacking gel buffer. Now the 5% stacking gel solution ((0.5M Tris-HCl, PH 6.8-2ml, 30% Acrylamide-0.8ml, 10% SDS-0.5ml, double distilled water -1.2 ml TEMED -0.015 ml 1.5% Ammonium per sulfate 0.5ml) was prepared and poured over the polymerized resolving gel, comb was inserted carefully. The gel slab was left undisturbed for 15 minutes, after polymerization comb was removed carefully and the prepared samples were loaded into the wells and gel was run at 60V.

Determination of molecular weights of the protein subunits separated on SDS PAGE:

To determine the molecular weight of the individual subunits of the protein, the relative mobility of the individual subunit was calculated by using the following formula.

$$\text{Relative mobility } R_m \text{ value} = \frac{\text{Distance travelled by individual subunit}}{\text{Distance travelled by the marker dye}}$$

Results

The electrophoretogram-1 represents the decrease in the intensity of gill protein subunits compared to control. Under exposure to lethal Cadmium Chloride, the intensity of gill protein subunits showed more decreased compared to the sub-lethal concentration. The Rm value (Table-1)of protein subunit 0.36 nearer to molecular weight 66,000 Daltons was absent in Lethal concentration of Cadmium Chloride and Rm value of protein subunit 0.71 in between molecular weights of 29,000 Daltons and 20,100 Daltons (Fig-1) was absent in both lethal and sub lethal concentrations of cadmium chloride compared to control. The electrophoretogram-2 represents the decrease in the intensity of brain protein subunits compared to control. Under exposure to lethal Cadmium Chloride, the brain protein subunits showed more decreased intensity in banding pattern compared to the sub-lethal concentration. The Rm values of 0.39, 0.43, 0.55 and 0.62 (Table-2) protein subunits in between molecular weight 66,000 daltons and 29,000 daltons (Fig-2) were completely disappeared in the lethal concentration exposed tissue samples.

Table 1: Changes in Rm values of protein subunits of the fish Cirrhinus mrigala (Hamilton) control gill, sub-lethal and lethal concentration of Cadmium Chloride for 96 h

Marker	Lane-1 Control	Lane-2 Sub-Lethal	Lane-3 Lethal
--	0.11	0.11	0.11
--	0.12	0.12	0.12
0.13	--	--	--
--	0.22	0.22	0.22
0.33	--	--	--
--	0.36	0.36	--
--	0.42	0.42	0.42
0.45	--	--	--
--	0.46	0.46	0.46
--	0.48	0.48	0.48
--	0.59	0.59	0.59
0.66	--	--	--
0.7	--	--	--
--	0.71	--	--
--	0.81	0.81	0.81
0.82	--	--	--
	0.86	0.86	0.86

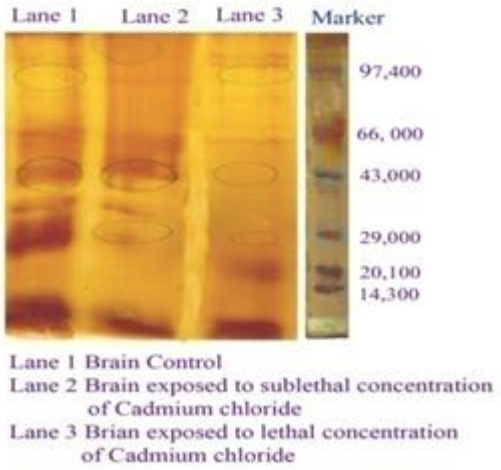

Fig 1: Changes in protein sub banding pattern in gill tissue of *Cirrhinus Mrigala* under exposure to Sub-lethal and lethal concentrations of Cadmium chloride for 96 h.

Table 2: Changes in R_m values of protein subunits of the fish *Cirrhinus mrigala* (Hamilton). control brain, sub-lethal and lethal concentration of Cadmium Chloride for 96 h

Marker	Lane-1 Control	Lane-2 Sub-Lethal	Lane-3 Lethal
--	0.11	0.11	0.11
0.13	--	--	--
--	0.16	--	--
--	0.25	0.25	0.25
--	0.3	--	--
0.33	--	--	--
--	0.35	--	--
--	0.38	0.38	0.38
--	0.42	0.42	0.42
0.45	--	--	--
--	0.47	0.47	--
--	0.54	--	--
--	0.60	0.60	0.60
--	0.73	0.73	0.73

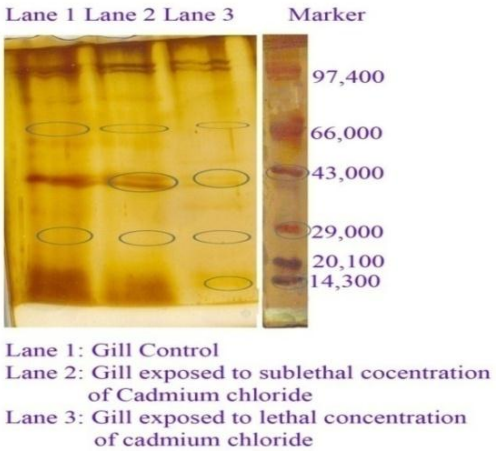

Fig 2. Changes in protein banding pattern in Brain tissue of *Cirrhinus Mrigala* under exposure to Lethal and Sub-lethal concentrations of Cadmium chloride for 96 h.

The electrophoretogram-3 represents the liver protein subunits of control, sub-lethal and lethal Cadmium chloride exposed samples. The intensity of protein sub units decreased in liver, compared to control. Under exposure to lethal concentration the intensity of liver protein subunits showed more decreased in banding pattern compared to sub-lethal concentration. The Rm value of protein subunit 0.47 (Table-3), molecular weight nearer to 43,000 Daltons (Fig-3) was absent in lethal concentration. Whereas Rm values of protein subunits of 0.16, 0.30, 0.35 and 0.54 were disappeared in both lethal and sub-lethal tissue samples. The electrophoretogram-4 represents the decrease in the intensity of muscle protein subunits compared to control. Under heavy metal Cadmium chloride lethal exposure, the tissue samples, of muscle protein subunits showed slight decreased intensity of banding pattern compared to the sub-lethal concentration and control exposures. The Rm values of protein subunits 0.15, 0.24, 0.32 (Table-4)subunits of control fish in between molecular weight, 97,000 Daltons to 66, 000 Daltons (Fig-4) were disappeared in both sub-lethal and lethal concentration samples.

Table 3: Changes in Rm values of protein subunits of the fish Cirrhinus mrigala (Hamilton) control Liver, sub-lethal and lethal concentration of Cadmium Chloride for 96 h.

Marker	Lane-1 Control	Lane-2 Sub-Lethal	Lane-3 Lethal
--	0.08	0.08	0.08
--	0.10	0.10	0.10
0.13	--	--	--
--	0.2	0.2	0.2
--	0.32	0.32	0.32
0.33	--	--	--
--	0.39	0.39	--
--	0.43	0.43	--
0.45	--	--	--
--	0.55	0.55	--
--	0.62	0.62	--
0.66	--	--	--
--	0.68	0.68	0.68
0.76	--	--	--
0.82	--	--	--
--	0.87	0.87	0.87

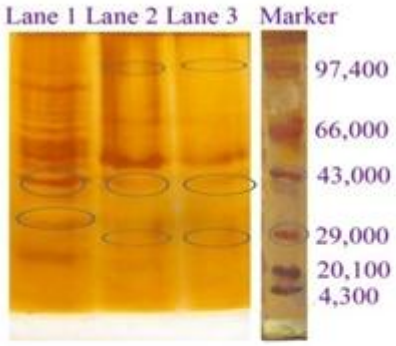

Fig 3: Changes in protein banding pattern in Liver tissue of *Cirrhinus Mrigala* under exposure to Lethal and Sub-lethal concentrations of Cadmium chloride for 96 h.

Table 4: Changes in R_m values of protein subunits of the fish *Cirrhinus mrigala* (Hamilton) control Muscle, sub-lethal and lethal concentration of Cadmium Chloride for 96 h.

Marker	Lane-1 Control	Lane-2 Sub-Lethal	Lane-3 Lethal
--	0.04	0.04	0.04
--	0.06	0.06	0.06
0.13	--	--	--
--	0.15	--	--
--	0.24	--	--
--	0.32	--	--
0.33	--	--	--
0.45	0.45	0.45	0.45
--	0.55	0.55	0.55
--	0.61	0.61	0.61
0.66	0.66	0.66	0.66
--	0.73	0.73	0.73
0.76	--	--	--
0.82	--	--	--
--	0.96	0.96	0.96

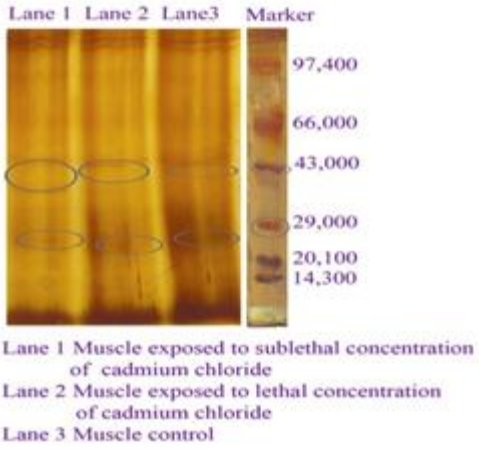

Fig 4: Changes in protein banding pattern in Muscle tissue of *Cirrhinus Mrigala* under exposure to Lethal and Sub-lethal concentrations of Cadmium chloride for 96 h.

The electrophoretogram-5 represents the changes in kidney protein subunits of sub-lethal and lethal concentration of Cadmium chloride exposed for a period of 96 h. The results showed a decrease in the intensity of kidney protein subunits compared to control. In the Cadmium chloride exposed tissue samples, the lethal exposed kidney protein subunits decreased intensity in banding pattern compared to the sub-lethal exposed tissue sample. The R_m value of protein subunit 0.42, molecular weight nearer to 43,000 Daltons (Fig-5) disappeared in sub-lethal and lethal concentrations. Whereas R_m values of protein subunits of 0.22, 0.30, 0.34 and 0.56 (Table-3) were absent in both lethal and sub-lethal tissue samples.

Table-5: Changes in R_m values of protein subunits of the fish *Cirrhinus mrigala* (Hamilton) control Kidney, sub-lethal and lethal concentration of Cadmium Chloride for 96 h.

Marker	Lane-1 Control	Lane-2 Sub-Lethal	Lane-3 Lethal
--	0.12	0.12	0.12
0.13	--	--	--
--	0.14	0.14	0.14
--	0.22	--	--
--	0.3	--	--
0.33	--	--	--
--	0.34	--	--
--	0.38	0.38	0.38
--	0.42	--	--
0.45	--	--	--
--	0.45	0.45	0.45
--	0.56	--	--
--	0.6	0.6	0.6
--	0.73	0.73	0.73
0.66	--	--	--
--	0.68	0.68	0.68
0.76	--	--	--
0.82	--	--	--
--	0.87	0.87	0.87

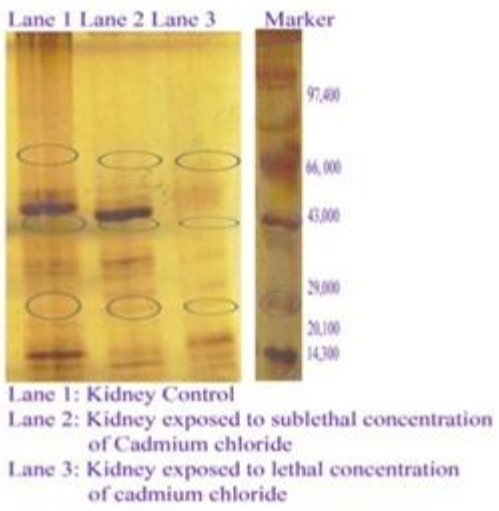

Fig 5: Changes in protein banding pattern iin Kidney tissue of *Cirrhinus Mrigala* under exposure to Lethal and Sub-lethal concentrations of Cadmium chloride for 96 h.

DISCUSSION

In the present study SDS polyacrylamide gel electrophoresis was performed for the tissues of gill, brain, liver, muscle and Kidney of Cirrhinus mrigala (Hamilton) exposed to sub-lethal and lethal concentrations of Cadmium chloride. When compared to control the protein subunits of gill, brain, liver, muscle and Kidney exposed to sub-lethal and lethal concentrations, the bands showed decrease in intensity. Among sub-lethal and lethal concentrations of cadmium chloride heavy metal exposed tissues. The bands showed more decrease in intensity (or) significant fading in lethal concentration exposed tissue samples than sub-lethal concentration. This indicates that lethal concentration of cadmium chloride may be more toxic than sub-lethal concentration. The changes in protein subunit band patterns may be due to change in the turn over (Synthesis /degradation) of various proteins.

In the gill tissue absent of protein subunit 0.36 effected by lethal concentration and the absent of protein subunit 0.71 effected by lethal and sub-lethal concentrations of cadmium chloride in the muscle. In the brain tissue protein subunits 0.39, 0.43, 0.55 and 0.62 were completely disappeared in the lethal concentration exposed tissue samples. It was suggested that the absent of protein subunits, toxic effect of heavy metal is lethal concentration of cadmium chloride. In the liver protein subunit 0.47 was absent in lethal concentration. Protein subunits of 0.16, 0.30, 0.35 and 0.54 were absent in both lethal and sub-lethal tissue samples. Absent of protein subunits in both concentrations sub-lethal and lethal shows heavy metal cadmium chloride is toxic to fish. In the muscle tissues protein subunits 0.15, 0.24, 0.32 were absent in both sub-lethal and lethal concentration samples. It was suggested that the absent of protein subunits in both cadmium chloride concentrations toxic to fish. In the kidney tissue protein subunit 0.42 was absent in sub-lethal and lethal concentration exposed sample. Protein subunits of 0.22, 0.30, 0.34 and 0.56 were absent in both lethal and sub-lethal tissue samples, because of toxic effect of heavy metal cadmium chloride.

The inhibition or activation of physiological activities by cadmium chloride is due to the interaction between the animal and the heavy metal. The stress induced biochemical changes can be described as secondary responses of the fish. According to Abou-Donia (1988), the biochemical analysis of DNA, RNA and protein are considered as markers in the toxicity study. Anees (1974) found that the total serum protein of Channa punctatus decreased significantly on exposing to some organophosphorous compounds. Kurbanova et al. (2004) reported that a decrease of the intensity of total protein accumulation and albumin concentration, and the increase of gamma globulin and peptidase activity which considered as adaptive reactions of the fish, Rutilus frisii kutum to the oil pollution. Patterson (1976) mentioned that the pollutants react with the cell nucleoproteins and nucleic acids and consequently affect the protein synthesis and cellular integrity. However, effects of toxicants on energy conservation by mammalian mitochondria have never been reported. In several toxicological models, movement disorders were linked to a dysfunction of the mitochondria.

Tripathi and Shukla (1990a,1990b) observed alterations in the Cytoplasmic protein pattern of fish Clarias batrachus by performing SDS-polyacrylamide gel electrophoresis of the cytoplasmic protein fractions of the liver and the skeletal muscle exposed to endosulfan and methyl parathion for 1 to 28 days. Munshi,(1999) stated that the fish Heteropneustes fossilis after exposure to malathion at sub-lethal concentrations for 24,48,72 and 96 hr, showed the formation of three low and four high mobility fractions and the disappearance of some protein fraction at different periods of exposure. Changes in protein sub units are regarded as important biomarkers of the metabolic potential of cells, as these play the main role in regulating the activities of cells. Their ratios also provide significant information about the way in which, mechanism, these contents regulate the multifaceted activities of cells.

CONCLUSION

The present study it was observed that the variations of protein patterns in Liver, brain, muscle gill of and kidney of the fresh water fish Cirrhinus mrigala (Hamilton) on acute exposure to sub-lethal and lethal concentrations of Cadmium chloride. In view of the importance of fish to diet of man, it is necessary that biological monitoring of the water and fish meat for consumption should be done regularly to ensure continuous safety of the fresh water food. Safe disposal of domestic sewage and industrial effluents should be practiced and were recycled to avoid these metals and other contaminants from going into the environment. The study showed a need for continuous pollution assessment study of aquatic organisms in fresh water body.

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