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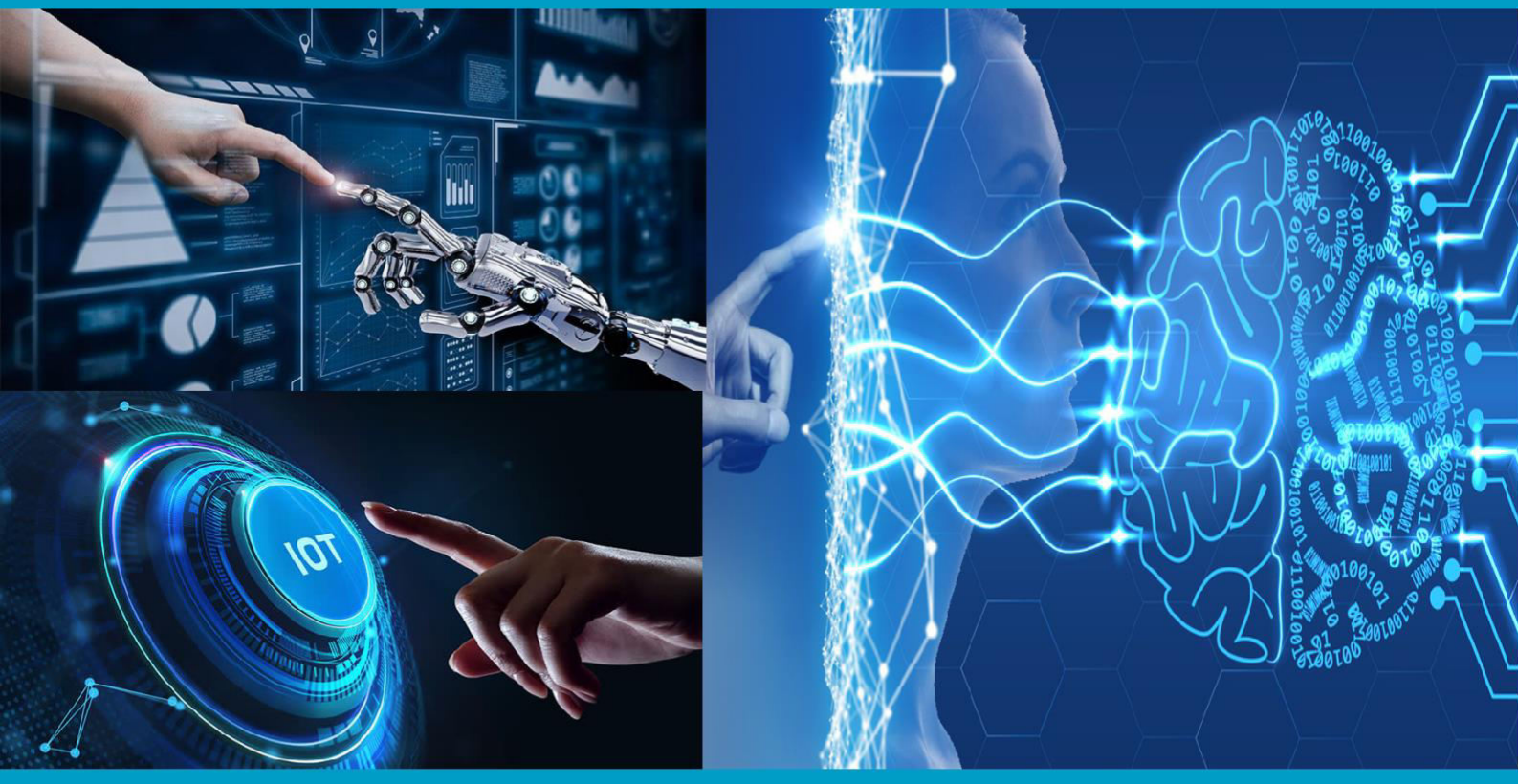

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Impact of Heavy Metals on SDS-PAGE Profile of Liver of *Cyprinus carpio*

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Abstract: The investigation used two sublethal dosage levels of 0.025 and 0.05 ppm of arsenic, mercury, nickel, and chromium separately as well as arsenic in combination with mercury, nickel, and chromium. After being exposed to various heavy metal treatments, liver protein extracts from *C. carpio* showed a clear pattern of protein profile alterations on gel electropherograms. With the subsequent deletion of some protein fractions, it led to the synthesis of certain new protein fractions in almost all of the treatments.

KEYWORDS: Heavy metal toxicity, SDS – PAGE, stress proteins, liver, *Cyprinus carpio*.

I. INTRODUCTION

Due to their persistence, water solubility, non-degradability, potent oxidizing properties, and strong affinities for numerous biological components, heavy metals are now referred to as the "devil in disguise" and are kept at the top of the priority list among water contaminants. They cause acute toxicity at higher dosages and cumulative harm over time at low ones. Fish physiological, histological, and biochemical activities are negatively impacted by heavy metals (Jain and Sharma, 2003; Jain and Mittal, 2004). They have hazardous effects on organisms at the molecular, cellular, and tissue level. Teratogenesis is a prime example of how cellular-level toxins disrupt reproduction, differentiation, and maturation. They primarily alter the cell membrane's permeability, which interferes with energy metabolism, and they also reduce the stability of the lysosomal membrane, which impairs cell functions by releasing a variety of hydrolases on a molecular level. According to Mizrahi and Achituv (1989), these metals' interactions with proteins can cause denaturation, precipitation, allosteric effects, or enzyme inhibition. They attach to nucleic acids, changing their shape in an irreversible manner. In fish, heavy metals also stimulate the production of proteins (Ali et al., 2003; Boone and Vijayan, 2002). According to research, a special family of proteins known as heat shock proteins has developed to protect against the possibility that proteins may become unstable or denatured under stress. Animals only use a group of molecules collectively known as heat shock proteins to tolerate stress. These proteins interact with several systems in pleiotropic ways that are controlled by the endocrine system. These HSPs play crucial functions in assisting fish to adapt to environmental change (Wali and Balkhi 2016),). The liver is the target and center for metabolism and may concentrate heavy metals (Farombi et al., 2007). This study thus aims to highlight changes in the protein profile of the liver of locally cultivated fish *C. carpio* after exposure to sub-lethal doses of some commonly found heavy metal pollutants in water, which would constitute important biomarkers as a clinical test for determining heavy metal toxicity in fishes.

II. MATERIALS AND METHODS

Cyprinus carpio weighing 90±15 gm were procured from local fresh water ponds and acclimatized in tank filled with well aerated water for seven days, then treated with As, Hg, Ni and Cr individually and As in combinations with others at 0.025 and 0.05 ppm concentrations in plastic tubs of 40 litre capacity. After 45 days treatment, the fishes were dissected and liver of the control and treated fish was analyzed .Samples were prepared by crushing 100 mg of tissue in 1 ml chilled phosphate buffer (0.1 M, pH 7.0) along with 50 mg insoluble PVP using pestle and mortal (rinsed with D.D.W. and dried) under cold conditions. The contents were then centrifuged at 10,000 rpm at 4^0C for 15 min in a refrigerated centrifuge. The supernatant containing the proteins was taken in Eppendorf tubes and pallets were discarded. The supernatant was stored at -20^0C.

Changes in number of proteins were studied for different treatments by SDS-PAGE, using discontinuous buffer system of Laemmli (1970). For molecular weight determination, a mixture of the marker proteins was also electrophoresed simultaneously in the same gel in the wells adjacent to sample wells.

After completion of electrophoresis, staining and background destaining, relative mobilities (Rm values) were calculated for each of the marker proteins and the resolved proteins by the following formula:

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$$\text{Rm value} = \frac{\text{Migration distance of protein band (mm)}}{\text{Migration distance of tracking dye (mm)}}$$

Rm value of marker proteins were plotted against log of molecular weights of marker proteins using semilogarithmic paper. Molecular weights of different proteins were estimated by matching their Rm values with appropriate point on the standard curve.

III. RESULTS AND DISCUSSION

The liver protein extracts of *C. carpio* revealed a total of ten protein bands in the control fishes of 25.1 to 169.8 kDa Mr (Table 1). Three bands of 100.0, 39.8 and 37.1 kDa Mr were dense over others. As at 0.05 ppm induced maximum number of fifteen protein fractions, with the synthesis of seven new fractions (158.4, 154.8, 147.9, 117.4, 109.6, 93.3 and 69.1 kDa Mr) and inhibition of two fractions in fishes. The similar alterations were observed in fishes treated with As at 0.02 ppm level, except the 154.8 and 93.3 kDa mr fraction. Three bands of 56.2, 44.2 and 29.5 kDa Mr were better expressed over control. Cr (0.05 ppm) treatment also induced synthesis of six new protein fractions. Except that of 144.5 kDa Mr, all other fractions were similar to As (0.05 ppm). It also induced deletion of two protein fractions one 153.1 kDa Mr similar to As (0.05) and the other was of 37.1 kDa Mr. Cr at 0.025 ppm induced almost similar alterations like Cr (0.05 ppm) except additional synthesis of 158.4 kDa Mr, resulting in total 13 protein fractions. One band of 56.2 kDa Mr protein was highly expressed while other two bands of 77.6 and 44.6 kDa Mr were also better expressed over the control. Expression of a protein fraction of 100 kDa Mr was reduced. Hg at both the doses induced synthesis of three additional protein fractions resulting in total thirteen protein fractions. Out of which two were similar in both the mercury treatments followed by deletion of a protein fraction of 153.1 kDa Mr. Ni (0.025) induced synthesis of a single protein of 50.1 kDa Mr followed by deletion of 39.8 kDa Mr fraction. Ni at 0.05 ppm level also caused similar alternation in Ni (0.025) treatment except synthesis a new protein fraction of 117.4 kDa Mr.

Gel electropherograms of *C. carpio* exposed to different combination of metals (Table 2) revealed that Hg in combination with As at both concentration levels induced alterations similar to As (0.05) except addition of a new fraction 144.5 kDa Mr, resulting in a total of sixteen protein fractions. Ni in combination with As also caused alterations similar to As (0.05) except synthesis of a new protein fraction of 50.1 kDa Mr. Similarly Cr in combination of As also induced same of the alterations similar to As (0.05) except synthesis of a new band of 154.8 and deletion of 37.1 kDa Mr protein fraction. This combination at 0.05 ppm level induced alteration similar to that at 0.025 ppm level except synthesis of a new protein fraction of 142.8 kDa Mr and deletion of 100.0 kDa Mr protein fraction resulting in total of fifteen protein bands. Alterations in band intensity were similar to that of As (0.05).

Since liver is the main detoxifying organ of the body and is reasonable to expect maximum toxic injury in this organ. Earlier studies with mercury and lead salts (Sastry and Gupta, 1978,a,b,c) have shown that liver is the most affected organ. Other most conspicuous changes in liver include vacuolation of cytoplasm of hepatocytes, pycnotic nuclei, connective tissue hardening and necrosis after mercury intoxication (Sastry and Gupta, 1978a). Hinton *et al.* (1973) showed scarring of the walls of blood vessels and formation of connective tissue septa in channel catfish treated with methylmercury. Pandey and Saxena (1990) also reported vacuolation in the hepatocytes and degeneration of cell membrane in the fingerlings of major carp, *L. rohita* exposed to sublethal concentration of copper.

The findings of the current experiments showed that protein fractions and their protein intensity profiles underwent clear qualitative changes. Mercury, a heavy metal, was discovered in earlier investigations (Suresh et al., 1991) to affect cellular metabolism, which resulted in altered protein synthesis machinery in C. carpio. The proteotoxic effects of heavy metals were mostly responsible for the alterations in protein profiles seen following heavy metal treatment. Due to stress brought on by metals in the effluent, Borgia et al. (2019) also noted the emergence or disappearance of protein fractions in the serum of C. carpio compared to control fish. When damage to proteins occurs, stress proteins commonly called as hsps (Heat shock proteins) are believed to be induced. It has been also known that stress proteins, also known as chaperones, prevent the reactive hydrophobic parts of the damaged proteins from forming non-specific complexes with normal cellular proteins (Weigant *et al.*, 1997). Thus, the alteration in the gel electrophoretic profiles or loss or gain of bands would possibly have some correlations with these stress proteins.

Apart from their protein damaging action, heavy metals have been reported to cause chromosomal damage (Bartoli *et al.*, 1991), increased DNAase activity (Joshi and Desai, 1988), decrease in DNA and RNA levels (Chaudhary, 2004). Therefore, the change of gel electrophoretic band profile might actually reflect damage in DNA or protein synthesizing system by heavy metal treatments.

Various authors (Boone and Vijayan, 2002; Tabche, 2002; Ali *et al.*, 2003; Chaudhary, 2004) reported induction of stress proteins by heavy metals. In this study, it was observed that metals are effective inducers of stress

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proteins, although the specific stress proteins induced could vary considerably. This was influenced by the type and dose of metal administered. This statement is exactly supported by Goering and Fisher, 1995 and Sanders *et al.*, 1996.This specificity in response makes it difficult to offer generalization regarding the induction of specific stress proteins by metals. These differences in protein induction might also reflect differences in the mechanisms of action by which specific metals elicit toxicity.

Heat shock proteins are the only molecular mechanisms that living organisms adopt to tolerate heavy metal stress, and these proteins have pleiotropic effects, interacting with multiple systems in diverse ways regulated by the endocrine system. Heat shock proteins are important in relation to heavy metal stress resistance and adaptation to the environment. Heat-shock proteins play an important role in regulating a range of effect or components, all of which contribute to survival under heavy metal stress by solving the problem of misfolding and aggregation, as well as its role as chaperones (Joseph et.al.2012). Based on the synthesis of stress proteins on exposure to heavy metals, SDS-PAGE profile study could constitute important biomarker as clinical test for determining heavy metal toxicity in fishes. Muhammada et al (2018) further reported the use SDS-PAGE protein profile for toxicological aspects, taxonomic studies and for population relationships. Osman et.al. (2010) also inferred protein electrophoresis as a sensitive tool for biomonitoring aquatic pollution.

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Conflict: There is no conflict of interest.

Table 1: Protein profile of liver of *Cyprinus carpio* exposed to different heavy metals

Sr. No.	Rm value	M.W. (k.Da.)	Control	Hg 0.025	Hg 0.05	Ni 0.025	Ni 0.05	Cr 0.025	Cr 0.05	As 0.025	As 0.05
1	0.003	169.8	+	+	+	+	+	+	+	+	+
2	0.030	158.4	-	+	+	-	-	-	+	+	+
3	0.038	154.8	-	+	+	-	-	+	+	-	+
4	0.046	153.1	+	+	-	+	+	-	-	-	-
5	0.053	147.9	-	-	-	-	-	-	-	+	+
6	0.07	144.5	-	-	+	-	-	+	+	-	-
7	0.16	117.4	-	-	-	-	++	+	+	+	+
8	0.19	109.6	-	-	-	-	-	+	+	+	+
9	0.23	100.0	++	++	++	++	++	+	+	+	+
10	0.26	93.3	-	++	-	-	-	+	+	-	+
11	0.34	77.6	+	+	+	+	+	++	++	+	+
12	0.38	69.1	-	-	-	-	-	-	-	++	++
13	0.48	56.2	+	+	+	+	+	+++	+++	++	++
14	0.53	50.1	-	-	-	+	+	-	-	-	-
15	0.59	44.6	+	+	+	+	+	++	++	++	++
16	0.63	39.8	++	++	++	-	-	++	++	-	-
17	0.66	37.1	++	++	++	++	++	-	-	++	++
18	0.76	29.5	+	+	+	+	+	+	+	+	+
19	0.83	25.1	+	+	+	+	+	++	++	++	++
Total number of proteins			**10**	**13**	**12**	**10**	**11**	**13**	**14**	**13**	**15**

Note - + : Least band intensity
++ : Medium band intensity
+++ Highest band intensity

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Table 2 : Protein profile of liver of *Cyprinus carpio* exposed to different combinations of heavy metals

Sr. No.	Rm value	M.W. (k.Da.)	Control	AS+Hg 0.025	AS+Hg 0.05	AS+Ni 0.025	AS+Ni 0.05	AS+Cr 0.025	AS+Cr 0.05
1	0.003	169.8	+	+	+	+	+	+	+
2	0.030	158.4	-	+	+	+	+	+	+
3	0.038	154.8	-	+	+	+	+	+	+
4	0.046	153.1	+	-	-	-	-	-	-
5	0.053	147.9	-	+	+	+	+	-	-
6	0.061	142.8	-	-	-	-	-	-	+
7	0.070	144.5	-	+	+	-	-	+	+
8	0.16	117.4	-	+	+	+	+	+	+
9	0.19	109.6	-	+	+	+	+	+	+
10	0.23	100.0	++	+	+	+	+	+	-
11	0.24	154.8	-	-	-	-	-	+	+
12	0.26	93.3	-	+	+	+	+	+	+
13	0.34	77.6	+	++	++	++	++	++	++
14	0.38	69.1	-	++	++	++	++	-	-
15	0.48	56.2	+	++	++	++	++	++	++
16	0.53	50.1	-	-	-	+	+	-	-
17	0.59	44.6	+	++	++	++	++	++	++
18	0.63	39.8	++	-	-	-	-	++	++
19	0.66	37.1	++	++	++	++	++	-	-
20	0.76	29.5	+	+	+	+	+	+	+
21	0.83	25.1	+	++	++	++	++	++	++
Total no. of proteins			**10**	**16**	**16**	**16**	**16**	**15**	**15**

Note - + : Least band intensity
++ : Medium band intensity
+++ : Highest band intensity

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