INVESTIGATION OF HSP70 GENE EXPRESSION IN KIDNEY, LIVER AND BRAIN OF CYPRINUS CARPIO

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Abstract
Western transfer and dodecyl sodium sulphate- polyacrylamide gel electrophoresis (SDS-PAGE) are two of the most widely used and beloved techniques in cancer research to identify proteins and glycoproteins. Cellular response to a damaging stressor or to unfavorable environmental factors is frequently linked to the induction of heat shock proteins (Hsps). The primary objectives of this study were to determine whether stress-induced Hsp70 could be used to monitor Cyprinus carpio exposure to different soil pollutants, to determine the specificity of pollutants in the kidney, liver, and brain tissues targeted and in Hsp70 induction, and to determine whether dose-response relationships could be established and whether the stress-response observed was specific. The most researched HSPs are HSP70, which is distinguished from the other HSPs by its high sensitivity, abundance, widespread expression, and association with all subcellular compartments. Due to its numerous roles in the equilibrium of all living things and its quick reaction to any chemical stressor, HSP70 is the focus of this study because it is effective in determining the processes involved in environmental pollution and contamination. According to their molecular weight of 70 kDa, heat shock proteins are categorized as HSP70. These proteins have kept their structural consistency from the simplest to the most complex species. They are a member of the chaperone family, which includes proteins with various structures and similar functions.

Keywords: *Cyprinus carpio*, Mercury, Lead, HSP70, Western Blotting, SDS, Gene expression.

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Introduction

In reaction to diverse stressful situations, cells release a family of proteins called heat shock proteins (HSPs), also referred to as heat stress proteins. (Johnston et al., 2018) Heat shock proteins (HSPs) are a suite of highly conserved proteins produced in all cellular organisms, among which HSP70 is ubiquitous and most studied protein family (Mohanty et al., 2018). Hsp70 can be induced by various environmental stresses (Gallant et al., 2017; Yang et al., 2020). HSPs are largely categorized into families according to their molecular weights. These families include the HSP100, HSP90, HSP70, HSP60, HSP40, and several smaller HSP families. Various environmental and pathological stresses, such as toxins, oxidative conditions, hypoxia, glucose deprivation, water deprivation, osmotic pressure, infection, and inflammation, can also result in high amounts of intracellular HSP formation. These stressors are in addition to thermal stress (Wang et al., 2017; Xu et al., 2017). HSPs can also carry out a wide range of maintenance tasks that are necessary for cell viability (Srivastava, 2002). HSPs play a role in physiological processes like embryonic development, gonadal development, and spermatogenesis as well as cellular functions like protein folding and transport, cell cycle regulation, and apoptosis under normal circumstances (Johnston et al., 2018).

The major heat shock protein synthesized by eukaryotic cells belongs to a family of 70,000-dalton proteins(HSP70). Of all of the Hsps families that have been studied, the 70 kDa protein family (Hsp70) has been most widely used as a biomarker due to its rapid and significant increase during a wide range of environmental stressors (Jonsson et al., 2006). Tissue-specificity of Hsp70 proteins was shown to occur both in vertebrates and

invertebrates. In fish, a number of studies have demonstrated that the expression of Hsp70 is quite variable in different types of tissues following heat shock exposure (Cara et al., 2005).

Fish that live in freshwater reservoirs can be utilised as selective bioindicators of trace metals because they not only collect metals in their bodies but also respond to water contamination by altering a number of essential physiological processes (Dobrowolski et al 2006) The growth rates of fish vary, and they are quite perceptive of their surroundings. In order to acquire information on fish performance, growth metrics might be used. Changes in morphometric characteristics, body form, and chemical and biochemical body composition are all related to fish growth. Fish growth is influenced by the physio-chemical properties of the water and often declines in contaminated waterways (Hansen et al 2002; Rowe 2003).

Stress brought on by physiological behavioral changes brought on by toxicant exposure might stunt growth (Hansen et al 2002). Fish growth rates reflect any changes in food consumption or energy output brought on by toxins. Young children are known to be particularly vulnerable to intoxication.
One of the most obvious signs of metal poisoning is the inhibition of larval growth, with cadmium and copper being the most potent growth inhibitors (Vosyliene et al, 2003). The majority of research on a particular metal's impact on fish focuses on exposure to that metal. However, contaminated water sources frequently have high concentrations of different metals. Chemical combinations can have additive, synergistic, or antagonistic effects. The aim of the paper is to investigate the amount of the HSP70 gene expressed by the fishes in different organs brain, kidneys, and liver when the fishes are exposed to heavy metals.

Materials and Methods

Western Blot Analysis For Hsp 70 Gene Expressions

Cell lysates prepared from control, mercury and lead treated kidney, liver and brain of Cyprinus carpio were subjected for western blotting or immunoblotting. Protein estimation was done by dye (dye from BIORAD) binding reactor on ELISA reader. 1 λ of total cell extract was added to 200 λ of sterile double distilled water followed by 15 λ of dye. Optical density (OD) at 590 nm was measured as an end point measure of color development. BSA was used as a standard to calculate the protein. 20mg of total protein from each sample was in 10% SDS PAGE (Towbin et al., 1979) transferred on to nitrocellulose membrane from AMERSHAM. Membrane after transfer was incubated in 1% blocking kit (Mouse/ rabbit, from Boehringer Mannheim, Cat. No. 1520 709) for 1 h at room temperature followed by 1 h incubation with primary antibody (HSP 70 monoclonal antibody from Stressgen Biotech Canada) with 0.5% blocking reagent. The blot was washed after primary antibody treatment for 5 minutes x 3 times with TBST buffer. Membrane again incubated in POD labeled secondary antibody diluted with 0.5% blocking reagent for 30 minutes at room temperature. The blot washed with TBST x 3 times and membrane was incubated in detection reagent (Luminol) for 60 seconds and exposed to X-Ray film.

Results

Physicochemical analysis of freshwater
The physical and chemical properties of the freshwater in which *C. Carpio* was found and analyzed for the presence of dissolved salts and toxic metals. The dissolved oxygen content was found to be 6.2 0.4 mg/l, with a neutral pH of 7.3 0.01. The total hardness of the water was determined to be 345 99 mg/l, whereas the free CO_2 concentration was calculated to be 2.1-0.12 mg/l. In the tested water, there were no residues of mercury or cadmium, though there were traces of calcium (81 88 mg/l) and magnesium (34 mg/l). In addition, the water sample contained high levels of sulfates and chlorides (Table 1). This test showed that *C. carpio* had not been exposed to mercury before the trial began.

Table 1. The physicochemical characteristics of water were analyzed by using standard methods (APHA, 1995 and 2005).

Parameters	Values
Dissolved Oxygen	6.2 ± 0.4 mg/l
pH	7.3 ± 0.01 m
Temperature	28 ± 2°C
Total hardness	345 ± 99 mg/l
Free CO_2	2.1 ± 0.12 mg/l
Ca	81 ± 88 mg/l
Mg	34 ± 0.0 mg/l
Hg	Nil
Sulphates	112 ± 0.9 mg/l
Chlorides	234 ± 22 mg/l
Cd	Nil
Specific conductance	2340 (Micro siemens/cm) at 2°C

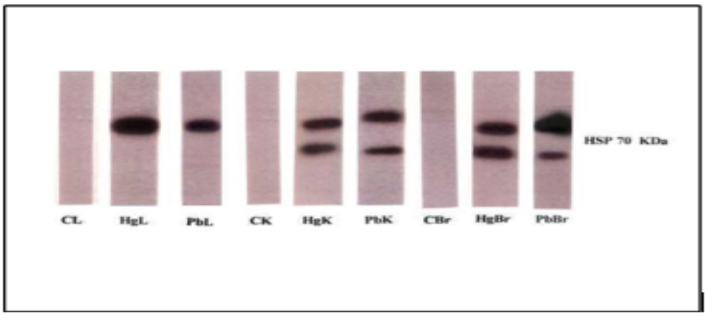
Electrophoretic Studies: Table 3. The HSP70 protein content of the liver kidney and brain was quantified Western Blotting Analysis.

Table: Number of protein bands present in different tissues of *Cyprinus carpio* exposed to Mercury and Lead

Duration(Days)	CL	HgL	PbL	CK	HgK	PbK	CBr	HgBr	PbBr
0	39	39	39	31	31	31	38	38	38
7	39	36	27	31	24	32	38	24	33
14	39	36	27	31	24	32	38	24	33
21	39	36	27	31	24	32	38	24	33
28	39	40	29	31	31	34	38	43	40
30	39	40	29	31	31	34	38	43	40

3.4 WESTERN BLOT ANALYSIS

HSP70 Gene Expression in the Control, Mercury and Lead treated tissues of Cyprinus carpio on 28th Day



Discussion

The gene expression, serum profiles, tissue histology, and bioindices of fish exposed to such metals are altered, and these changes serve as general health biomarkers. The heavy metals (Ni, Cd, and Cr) accumulated in water and fish tissues, were beyond the permissible limits defined by the Central Pollution Control Board/World Health

Organization. Accourdimg to Sadiya et al ,2020 Metallothionein (MT) and glutathione peroxidase (GPX) genes expression patterns highlighted the metal-specific exposure of fish. Fish are subject to a variety of stressors in the water, with heavy metals being the most commonly reported one (Banday et al., 2019). The constant accumulation leads to metabolic and genetic changes, and they are typically hazardous at larger concentrations.

SDS strives to achieve highly resolved separation of complex protein combinations. This method denatures the protein that will be exposed to electrophoresis. Since the protein fraction expression on the protein profile is significantly lower on the seventh day than it is on the eighth, the protein fraction expression is directly inversely related to the number of days. In order to determine the number of protein bands expressed in different tissues of Cyprinus carpio exposed to mercury and lead as the number of days rose, electrophoretic research was conducted. The difference between the 27 bands for lead and 36 bands for mercury on the seventh day and the 29 bands for lead and 40 bands on the thirty-first day suggests that as the number of exposure days to lead and mercury rises, so do the number of protein bands that are expressed. Using gel electrophoresis, a mixture of proteins is categorized by kind and molecular weight in the Western Blotting method. The results are then put into a membrane, which results in the formation of a band for each protein. Two HSP70 gene bands were found in Cyprinus carpio tissues after exposure to lead and mercury. Two genes may be seen on the plate on the 28th day, when the HSP70 gene began to express itself due to antigenic similarities and polypeptide variances. Because the liver is an organ that detoxifies the body, the buildup is lessened. As a result, the gene's strength greatly reduces as accumulating strength does. The brain is neurosensitive, while the kidney is the organ of excretion. Zang et al 2018 proved, HSP70, one of the most sensitive proteins that are generated under stress conditions and important for normal cell function, is upregulated in response to cadmium exposure in animals as a result of the hepatotoxicity that is brought on by cadmium exposure. According to the investigations, after being exposed to cadmium through water, P. olivaceus dramatically increased hepatic heat shock protein 70 (Deok-Chan Lee,2022)

Conclusion

Fish physiology is affected by heat shock protein genes in a number of ways, including growth and aging, stress physiology and endocrinology, immunology, environmental physiology, stress tolerance, and acclimatization (Basu et al. 2003). The reaction of the HSP gene can change depending on the tissue, different HSP families, and stresses. Season, developmental stage, and species can all affect how sensitively the Hsp gene is expressed. Prior research on fish examined the expression of the Hsp gene after bacterial infection. The investigations, however, mostly focused on Hsp90 and Hsp70. In the current communication, a variety of Hsp genes are investigated to determine their potential function in immunity in a comparative manner, and the findings regarding the expression of the Grp78 gene in liver tissue highlighted the crucial roles that these molecules played during bacterial pathogenesis. The Hsp90 gene appeared to be important in bacterial immunity, and fish virus infections were associated with higher expression levels of the gene.

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