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Achieving SDG'S in Bangladesh: Fish Stress Protein as Biomonitoring Tool for Sustainable Management of Water

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Abstract Water is working in a very significant role in all the sectors of sustainable development and therefore being responsible for numerous global and economic crises. Need exists for rapid means of assessing the "health" of water bodies in Bangladesh. This study aims to assess health indicator for aquatic lives. Nile tilapia (*Oreochromis niloticus*) were studied in the laboratory tanks of constant temperature, 28°C and were treated with $K_2Cr_2O_7$ and *Psedumonas*, commonly found stressor in the water bodies of Bangladesh. Test animal was used to obtain tissue for conducting SDS-PAGE. SDS-PAGE was applied to protein samples to linearize proteins. A dominant band of 100 kDa was found in the brain, gill and intestine after c exposure. Presence of 100 kDa suggests that HSp 100 can be the ideal stress marker. The present study also indicates that the use of stress protein for biomarker is easy to use and sensitive for managing water quality to achieve SDG in Bangladesh.

Keywords: *HSP100, stress protein, biomonitoring, environmental stressor, water management*

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1. Introduction

Water is working in a very significant role in all the sectors of sustainable development and therefore being responsible for numerous global and economic crises. [1]. So, in hope of all-out solution of these water issues, a unique step should be added in the SDGs. Besides, due to its interconnection with other global issues, a worldwide goal concerning water issues is being a must to put in effect some effective vows and coordinated actions to every water issue to achieve global development goals. Water issues are getting much worse and alarming nowadays. [1]. Interconnections among global issues like water, social and economic issues are getting more alarming with the help of rapid population growth, change of land usages, rapid unplanned urbanizations, and aftermath of climatic degradation and pollution of water simultaneously.

Reasonably, water security has aroused the necessity of the intersection of hydrology, ecology and society as well. [1]. Huge bulk of waste water from domestic and industrial areas carried by their surrounding water bodies like rivers and lakes is getting discharged into the seas which is frequently polluting waters as a whole. These contamination causes the most of all heavy metal contaminations. [2] Consequently, heavy metals get into fish body from those contaminated waters and alter the organisms. These contaminants not only damage the organisms but also get deposited into aquatic affecting bioconcentration, bioaccumulation and food chain system. Consequently, these contaminants can threaten the health of mankind. [2] Fishes maintain their normal metabolism by taking metal from water, foods and sediments. But a high concentration of heavy metals caused by water pollution is causing toxic effects in fish world. [2]

Now a day, appraisal of water quality is based only on chemical measurements and analyses of the water itself through early detection of changes in the quality of water resources impacted by anthropogenic contaminants along with microbial infestation. But a need has always been existed for a rapid means of assessing the "health" for sustainable management of water bodies in Bangladesh. A reliable, field applicable method with a valuable tool which determines stress levels in fish could provide resource managers to determine the severity of health of water for immediate management. This is possible with the use of biotests or biomarkers, e.g. investigations of the induction of heat shock proteins (proteotoxicity evaluate). [3] Heat shock protein (HSP) genes are highly conserved as well as are constitutively expressed in all tissues in response to stress. Stress disrupts homeostasis, the regular conformation of proteins demanding the aid of HSP to regularize the effect. In fish, stress could be due to any minor change in the environment [4]. Fish are exposed to many kinds of stressors such as microbial infection, toxic

exposure, traumatic damage, radiation, or nutritional deficiency. [2]Therefore, the modulation of HSP genes is more apparent in fish and can be studied as molecular biomarkers of stress because under adverse physiological conditions, there is a notable elevation of these protein. This will further indicate the supervision for management of water bodies. [3]

2. Materials and Methods

2.1. Sample

Nile tilapia (*Oreochromis niloticus*) were studied in the laboratory tanks of constant temperature, 28°C. The oxygen content in the water was maintained throughout the experiment by an aerator to prevent oxygen to act like a limiting factor and the other parameters are shown in Table 1.

Table 1. Water quality parameters

Parameter	Range
Dissolved Oxygen	6.75mg/l
Free CO2	43.6mg/l
pH	6.4
Alkalinity	145ppm
Hardness	100ppm
Nitrate	0.60

The fishes were fed with pelletized meal. This meal was contained with groundnut oil cake, Bengal gram powder and rice bran. Acclimatization continued for a period of 15 days prior to the initiation of the experiment at laboratory conditions. Fish of both sexes were used without discrimination. Table 2 shows length of the fish.

Table 2. Length of the fish

Parameters	Range	Mean
Length (cm)	3-6.9	4.95
Weight (g)	5.5-9.8	7.65

2.2. Heat Shock Exposure

Next to acclimatization, fish were relocated to a similar tank in which water previously heated up to 34 C, and were left for two hours. Then, the fish were moved back to the tank of normal temperature and were left for two more hours. Blood samples were collected to monitor glucose level using glucometer and Fish were anaesthetized by putting on the dry ice then dissected to obtain brain, liver, heart, muscle, intestine and gill. Tissues were kept directly with dry ice then keeping under -70°C for the next analysis.

2.3. Exposure to Biological Stressor

To initiate bacterial infection caused by *Psedumonas* in fish, the required number of cfu/ml in water is more than 1.5×10^3 to 8.6×10^3. Before applying pathogens, it was essential to quantify the number of living pathogens that could infect fish. For this purpose, some of the pathogenic bacteria were incubated in nutrient lysogeny broth to take the comparative optical density. This optical density helped to measure the amount of pathogen should be applied. Table 3 gives information amount of bacterial cells at specific optical density.

Table 3. Required number of *Psedumonas* to initiate bacterial infection

Sample No.	OD after 1hr 30min	No of cfu /ml	OD after 5hr 30min	No of cfu/ml	OD after 8hr 30min	No of cfu/ml	OD after 24hr/2	No of cfu/ml
2	0.03	2.6×10^6	0.396	2.4×10^8	0.885	7.8×10^8	0.696	6.7×10^{12}
3	0.127	2.5×10^6	0.419	2.16×10^8	0.628	5.8×10^8	0.581	1.7×10^{12}
5	.001	4.5×10^5	0.023	2.88×10^7	0.156	3.6×10^8	0.706	5×10^{12}
6	.011	2.6×10^6	0.429	2.016×10^8	0.605	4.4×10^8	0.617	6×10^{12}
8	.002	2×10^6	0.410	3.04×10^8	0.556	7.4×10^8	0.643	7.6×10^{12}

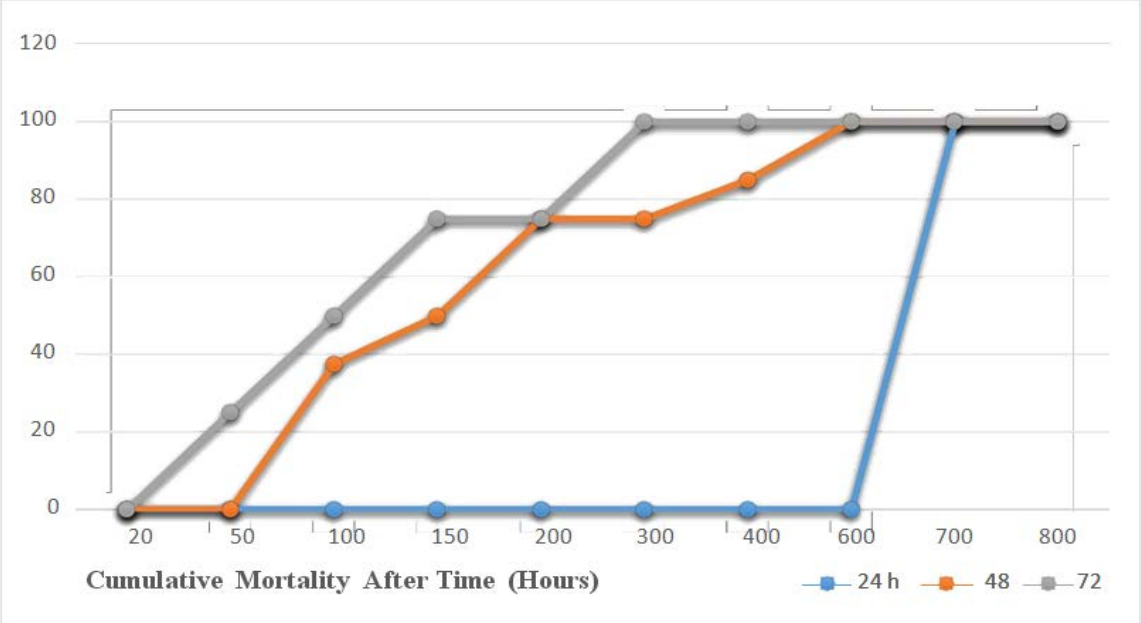

Figure 1. Cumulative mortality rate

2.4. LC_{50} Values at 24h, 48h and 72h for Singi Fish *(Heteropneustes fossilis)*

To calculate LC_{50} value, a trial was done. The trial was done within 50-600 ppm concentration range of $k_2Cr_2O_7$. The purpose of this trial was to test the toxicity of $K_2Cr_2O_7$ and to measure the range of LC_{50} values of $k_2Cr_2O_7$. The final trial was used to measure the LC_{50} values at 48h and 72h as accurately as possible. The LC_{50} values obtained are given in Table 4. Cumulative mortality rate is shown in Figure 1.

Table 4. LC50 values for K2Cr2O7 at 48h and 72h for Singi fish (*Heteropneustes fossilis*)

Time of Exposure(Hours)	LC50 Values for K2Cr2O7 (ppm)	LC50 Values for Cr (ppm)
48	150	26.51
72	120	21.21

2.5. Homogenization

Homogenized tissues

Tissues from fish were homogenized in 1ml tris-saline (1% SDS) by glass polytone. The homogenate is heated in water bath at 90°C for 5 minutes, and then centrifuged at 14,000 rpm for 10 minutes. The supernatant was obtained and kept in refrigeration until use.

2.6. SDS PAGE

To attain proteins of interest, gel concentration (%T) was selected according to the expected protein size (>100 kDa). Therefore A 10% separating gel was used, assembling distilled water, lower gel buffer along with 10% SDS and acrylamide solution. 10 ml of stacking gel mix was sufficient, however for the sake of accuracy20 or 30 ml was prepared. Excess was rinsed and tossed into a wastebasket after it polymerized. The sample was then carefully loaded onto the wells using a micropipette, one sample to one well by Hamilton syringes and the amount was limited to 10 µl. The gel was run at 100 volts. In this condition, the electrophoresis apparatus was kept undisturbed for at least 1 hour and 45 minutes for the smaller unit, until the tracking dye (bromophenol blue) reaches to the gel bottom. A commonly used stain for detecting proteins in polyacrylamide gels is 0.1% Coomassie Blue dye in 50% methanol, 10% glacial acetic acid. Excess dye was washed out by 'de-staining' with acetic acid, also with agitation For more efficiency, de-staining been done in two steps: starting with 50% methanol, 10% acetic acid for 1-2 hours. The first solution shrinks the gel, squeezing out much of the liquid components and the gel swells and clears in the second solution.

Later 7% methanol, 10% acetic methanol were used to finish. Properly stained/de-stained gels should display a pattern of blue protein bands against a clear background. The gels was dried down and photographed for analysis and documentation. The original dye front, consisting of bromophenol blue dye, disappeared during the process. In fact, bromphenol blue acted as pH indicator which turns light yellow under acid conditions, prior to being washed out.

3. Result

Blood glucose level was found to be higher for treated fish ranging from 2.39 at 24 hour to 9.2 at 72 hours with increasing concentration of $K_2Cr_2O_7$ (Table 5). After finding the glucose level elevated and the fish were stressed, they were sacrificed and the stress protein was identified. A dominant band of 100kDa was found in the gill, brain and intrstine after each chemical exposure. But was absent in any other organ every time when treated chemically.

Table 5. Changes in Glucose level by $K_2Cr_2O_7$

Conc. of $K_2Cr_2O_7$ (ppm)	24 h	48 h	72 h
Control (0)	2.39	2.78	3.83
20	3.27	3.58	4.6
50	4.3	4.67	4.88
100	5.15	5.65	5.96
150	6.18	6.3	6.88
200	7.15	7.53	7.69
300	7.39	7.68	8.03
400	8.35	8.98	8.98
600	8.7	8.96	9.2

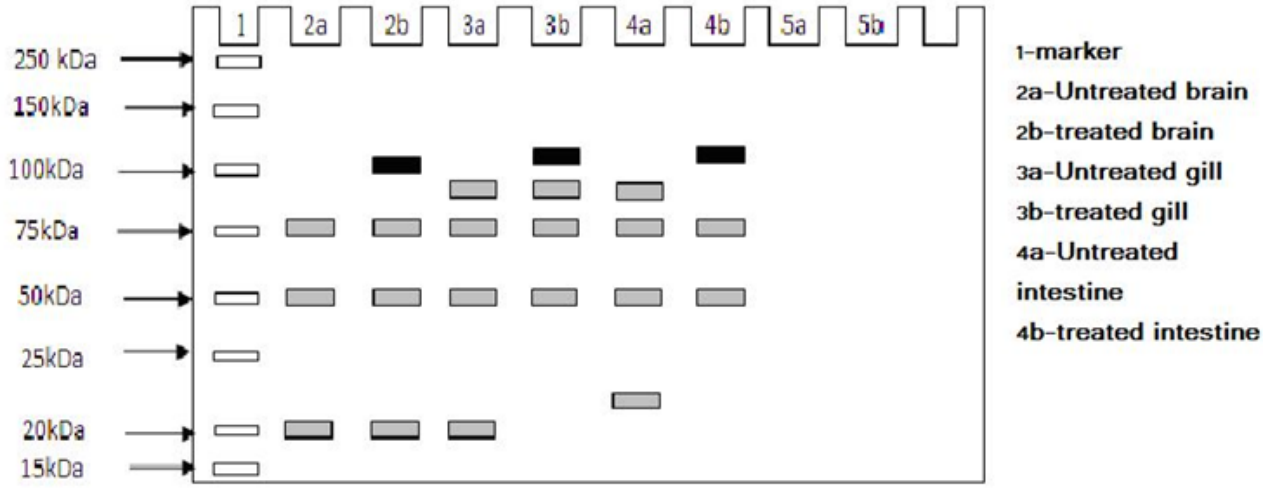

Figure 2. Banding pattern is shown in the zymograph

4. Discussion

There have been several efforts to validate the use of the hsp response as an indicator of stressed states in fish. [5] (Iwama 2004). Stress proteins mitigate the impact of stress in individuals and these mechanisms are becoming well understood at the level of model proteins, but less is known at the level of the cell, tissue, organ and whole organism. [6]. (Feder M.E, Hofmann, G.E. (1999). Therefore studies of stress protein response in organs with high metabolic activity, like brain,kidney and gills, are of higher interest. This provides the first evidence that Hsp100 in both brain,gill and intentine. And that certainly acts as a biochemical marker linked to proteotoxicity in Nile telapia fish. After the both biological and chemical treatment, the gills exhibited presence of the Hsp100 levels in treated fish and same was found for brain and kidney. The induction was not limited to fish, but higher expression was also reported in infected crabs, clams, and shrimps as well during Gram-negative bacterial exposure [7,8,9]. Therefore, the present study points out only the initial but pertinent information on aspects of the response of fish to environmental stresses. This result encourages further research in this topic of environmental stress physiology since long term studies in the field suggested seasonal changes in heat shock response of fish [10].

Biochemical responses serve as sensitive indicators of exposure to contaminants and or sub lethal stress. Thererore, synthesis of HSPs has the potential to act as a significant indicator for water management. Plasma cortisol concentration or haematocrit, is highly vulnerable to handling and sampling effects. The results of this research suggest the use of stress proteins as biomarkers may serve as an indicator for water management in situations where the response to stress is chronic and it is in turn make it possible to prevent the biological consequences of exposures which affect organismal or higher organizational levels. Though there is a chance of co relation between stress response and intensity of the stressor however, continuous exposure to severe environmental stress may lead to death results expressing the effects at the organismal level instead of stress protein response. Therefore, data from the current experiment suggest that the ideal stress marker may be met by examining the accumulation of HSP 100 in tissues. This indication is presented after exposure to environmentally biological and chemical contaminants. The present study also indicates that the use of stress protein as biomarker is very easy to use, practical, rapid, and sensitive for managing water quality.

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