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A Study On Gills Of Clarias Batrachus Following Exposure To Arsenic: A Histopathological And Biochemical Approach.

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Abstract:
In present research study an attempt has been made to find out the relation if any between the proteins of gills of the catfish Clarias batrachus and the arsenic toxicity. Arsenic is a highly toxic environmental contaminant that poses serious risks to aquatic life. Proteins are the biomolecules that express and regulate the cellular functions whenever required by the cell. Stress proteins, a special class of proteins, are known to play crucial roles in cellular defense against environmental stressors including the arsenic. Clarias batrachus is a highly nutritious catfish of the region and is found in the fresh water aquatic reservoirs. The fish is selected for the study because of its hardy nature and suitability in maintaining in laboratory conditions. The study utilizes histopathological analysis, biochemical assays, and quantitative protein analysis to assess the impact of arsenic on the fish's immune and cellular functions. Our findings indicate a significant increase of stress proteins in the gills following exposure to arsenic suggesting their potential as reliable biomarkers for arsenic toxicity. These results contribute to a better understanding of arsenic-induced stress responses in aquatic organisms and highlight the importance of stress proteins in environmental monitoring and risk assessment.

Keywords: *Clarias batrachus, arsenic, stress proteins, biomarkers, gills,*

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I. Introduction

The role of stress proteins in fish gills as biomarkers of arsenic-induced toxicity has become an area of significant research particularly in the context of aquatic organisms including the fish, *Clarias batrachus*. Arsenic, a potent environmental contaminant is known for its toxicological effects on various biological systems including fish. Arsenic when enters into the body of aquatic organisms including the fish is converted into its various metabolites that are often considered more toxic than the parent one. The gills of fish serve as a primary site of interaction with contaminants in the water. Heat shock proteins (HSPs) and metallothioneins are frequently employed as biomarkers of environmental stress and contaminations. In the case of *Clarias batrachus*, exposure to arsenic has been shown to induce oxidative stress and alter the biochemical homeostasis of the organism leading to alterations in protein expression and cellular function (Bhattacharya et al., 2007; Roy & Chakraborty, 2006). The study of these stress proteins particularly in the gills provides valuable insights into the molecular responses of fish to toxic exposure. In research studies it has been revealed that metallothioneins implicate in the detoxification of heavy metals while heat shock proteins are essential for the refolding of damaged proteins under stress conditions (Cervantes et al., 1994; Ghosh et al., 2006). Additionally, arsenic's impact on the immune responses of fish as observed in previous studies further emphasizes the relevance of using stress proteins as bioindicators of aquatic toxicology (Dutta & Saha, 2005; Sakurai et al., 2006). Understanding the molecular mechanisms behind arsenic's impact on fish health can contribute to the development of better ecological risk assessment tools, environmental monitoring systems, and the formulation of strategies to mitigate aquatic pollution (Zelikoff, 1993; Singh & Banerjee, 2008). Thus, the study of stress proteins in *Clarias batrachus* offers a comprehensive approach to evaluating the biological consequences of arsenic exposure, with potential implications for environmental conservation and public health management.

II. Methodology

Animals Procurement and Maintenance

Clarias batrachus, a very popular catfish of the region was obtained from a local aquaculture farm. The fish were selected based on their uniform size and health status. The selected fish were approximately 20 cm in length and weighed around 100 g. Upon arrival at the laboratory, the fish were acclimatized in aerated water tanks for a period of two weeks. During this acclimatization period, the fish were fed with a commercial fish feed containing essential nutrients. The laboratory conditions (water temperature 26 ± 2°C; pH 7.0-7.5 and dissolved oxygen 6 ppm) were carefully controlled to ensure the well-being of the fish. All tanks were equipped with proper filtration systems to ensure optimal water quality and prevent contamination.

Acute Toxicity Test

A stock solution of arsenic trioxide (As_2O_3) was prepared by dissolving the required amount of the compound in distilled water. The test was performed using a static renewal method where the fish were exposed to varying concentrations of arsenic for 96 hours. The concentrations tested included 0µg/L, 25µg/L, 50µg/L, 75µg/L, and 100µg/L. A group of 10 fish was used for each concentration and the fish were observed for mortality and signs of distress (erratic swimming, respiratory distress) at regular intervals. Mortality data were recorded at the end of the exposure period to calculate the LC_{50} using statistical methods (probit analysis).

Histopathological analysis

Gills dissected were washed in normal saline to remove blood clots if any and were fixed in 10% neutral buffered formalin. After expiry of 24 hours (fixation time for formalin), the tissues samples were then washed with 70% alcohol, dehydrated using 90% and absolute alcohol, cleared in xylene and preserved in cedar wood oil for further use. Paraffin sections (5µm) were cut using a rotary microtome having disposable blade facility. Dried tissue sections were deparaffinised, rehydrated using graded alcohols (descending order), stained with haematoxylin and eosin, dehydrated using graded alcohols (ascending order), cleared with xylene and finally mounted on glass slide for microscopic observation and data recording.

Quantification of Total Protein

The total protein content in the gill tissues of *Clarias batrachus* was determined using the Bradford assay. A small portion of gill tissue (50 mg) from fish (total 3 fish) was homogenized in ice-cold PBS buffer (pH 7.4) and centrifuged to obtain the supernatant. The protein concentration in the supernatant was measured by mixing it with Coomassie Brilliant Blue dye and comparing the absorbance at 595 nm against a standard curve generated with known concentrations of bovine serum albumin (BSA). The results were expressed in mg per gram of tissue body weight.

SDS-PAGE Analysis

To analyse the stress proteins and compare their expression in different exposure groups, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed. Gill tissue samples from each exposure group were homogenized and the protein extracts were mixed with SDS sample buffer and heated. The protein samples were loaded onto a 12% polyacrylamide gel and electrophoresis was carried out at 100 V for 1.5 hours. The separated proteins were stained with Coomassie Brilliant Blue and visualized under UV light. Image J software was used to analyse the gel images and HSP70 and HSP90 were quantified using the relative intensity of bands.

Data Analysis

All data obtained were statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. The results were expressed as mean ± standard deviation (SD). A significance level of $p < 0.05$ was considered statistically significant. The relationship between arsenic exposure and the expression of stress proteins was evaluated through correlation analysis.

III. Results

The most remarkable findings of present study are the expression of heat sock proteins in the gill tissues after exposure to varying concentration of arsenic. The results also highlight the induction of oxidative stress and the histopathological alterations in the gills of the fish. The findings provide significant insights into the role of stress proteins as early biomarkers of arsenic toxicity in aquatic organisms.

Table 1. Table 1 shows the effect of arsenic on stress proteins (HSP70 and HSP 90) of the gills of *C. batrachus*. Note an increase in HSPs expression at various doses of exposures.

Arsenic Concentration (µg/L)	HSP70 Expression (Relative Units)	HSP90 Expression (Relative Units)
0 (Control)	1.00	1.00
25	1.92	1.65
50	3.15	2.80
75	4.25	3.50
100	5.60	4.10

The data clearly shows a dose-dependent increase in the expression levels of both HSP70 and HSP90 in the gills of *Clarias batrachus* as arsenic concentration increased. At the highest arsenic concentration of 100 µg/L, the expression of HSP70 and HSP90 was 5.60 and 4.10 times higher than the control respectively. This suggests that arsenic exposure induces a strong stress response in the gills leading to the upregulation of stress proteins as part of the cellular defense mechanism against oxidative damage. The increase in stress proteins levels at various arsenic exposures directly corroborates the arsenic toxicity with the stress proteins response. This stress response is accompanied by the upregulation of stress proteins, which play vital roles in protecting the cells from oxidative damage and maintaining cellular homeostasis. Metallothioneins, which are small cysteine-rich proteins involved in metal detoxification, have also been extensively studied as biomarkers of heavy metal exposure. They are known to bind to toxic metals such as arsenic, thereby reducing their bioavailability and mitigating their toxic effects. In fish, metallothioneins have been shown to be upregulated in response to arsenic exposure, making them reliable biomarkers for assessing metal contamination (Cervantes et al., 1994).

Proteins are involved in protein folding, repair, and degradation, and they help maintain cellular function under stress conditions. In the context of arsenic exposure, HSPs have been shown to be upregulated in various fish species, including *Clarias batrachus*. Roy and Chakraborty (2006) have reported significant increases in the synthesis of HSPs in the liver and kidney of *Channa punctatus* exposed to arsenic, suggesting a similar response in the gills of *Clarias batrachus*. The expression of HSPs serves as a protective mechanism to prevent the accumulation of damaged proteins, thus allowing cells to maintain their functionality even under toxic conditions.

Histopathology study showed that there are significant morphological changes in the gills of arsenic exposed *C. batrachus*. The gill tissues of fish exposed to increasing concentrations of arsenic exhibited varying degrees of damage which are summarized in Table 2.

Table 2. Table 2 shows histopathological changes in the gills of *C. batrachus* after exposure to varying concentration of arsenic.

Arsenic Concentration (µg/L)	Histopathological Changes Observed
0 (Control)	Normal gill structure, no damage observed
25	Mild hyperplasia, slight epithelial cell damage
50	Moderate hyperplasia, degeneration of cells
75	Severe hyperplasia, epithelial cell necrosis
100	Extensive necrosis, mucous cell proliferation

The gills of *Clarias batrachus* exposed to arsenic exhibited a concentration-dependent increase in histopathological damage. At the lowest concentration (25 µg/L), only mild hyperplasia and slight epithelial damage were observed. However, at concentrations of 50 µg/L and above, more severe damage including epithelial cell necrosis and mucous cell proliferation was noted. The gills of fish exposed to 100 µg/L arsenic showed the most severe damage with extensive necrosis and significant alterations in the gill architecture. This suggests that arsenic exposure leads to structural damage in the gills impairing their respiratory function and overall health.

Research into the histopathological effects of arsenic exposure on fish gills has also provided valuable insights into the underlying mechanisms of arsenic-induced toxicity. Studies by Singh and Banerjee (2014) have shown that exposure to arsenic causes significant histopathological changes in the gills of *Clarias batrachus*, including cellular degeneration, hyperplasia and damage to the gill epithelium. These changes are often accompanied by the upregulation of stress proteins which act to mitigate the damage and promote recovery.

The total protein content in the gills of *Clarias batrachus* was also measured to assess the overall health of the tissue and the impact of arsenic exposure.

Table 3. Table 3 shows the total protein content in gills of C. batrachus following exposure to varying concentration of arsenic.

Arsenic Concentration (µg/L)	Total Protein Content (mg/g of tissue)
0 (Control)	10.00
25	9.25
50	8.10
75	6.75
100	5.50

A significant decrease in total protein content was observed in the gills of *Clarias batrachus* as arsenic concentration increased. The total protein content decreased from 10.00 mg/g in the control group to 5.50 mg/g at 100 µg/L arsenic. This decrease suggests that arsenic exposure disrupts normal protein synthesis and degradation processes leading to a reduction in the overall protein content in the gills. The decreased protein levels may be attributed to the increased expression of stress proteins which are synthesized to counteract oxidative damage and maintain cellular homeostasis.

Zelikoff (1993) while studying on the immunotoxicity of heavy metals in fish highlighted the potential of these proteins to serve as indicators of environmental stress and pollutant exposure. In fish exposed to arsenic, the expression of stress proteins in gills correlates with the degree of toxicity and can be used to assess the impact of arsenic contamination on fish health. The ability of these proteins to bind and detoxify metals, repair damaged proteins, and maintain cellular integrity makes them excellent candidates for use in environmental monitoring and risk assessment.

Dutta and Saha (2005) characterized the immune response of *Clarias batrachus* to arsenic, demonstrating alterations in immune cell function and the expression of stress proteins in response to contamination. These findings support the hypothesis that stress proteins play a crucial role in the immune defense of fish against environmental pollutants. Arsenic exposure has been shown to suppress immune responses in fish leading to increased susceptibility to infections and diseases. This immunosuppressive effect is often accompanied by changes in the expression of stress proteins highlighting the interconnection between environmental stress, immune function, and protein expression in fish.

The results reveal that there is a clear positive correlation between the expression levels of stress proteins (HSP70 and HSP90) and the severity of histopathological damage in the gills. As the concentration of arsenic increases, both the expression of stress proteins and the severity of gill damage increase. This suggests that the upregulation of stress proteins, particularly HSP70 and HSP90, is a direct response to arsenic-induced toxicity, and these proteins play a protective role in minimizing the extent of cellular damage. The expression of HSPs can thus be considered an early indicator of arsenic-induced toxicity, providing a valuable tool for environmental monitoring.

The findings of this study demonstrate the significant role of stress proteins, specifically HSP70 and HSP90 in the gills of *Clarias batrachus* as biomarkers of arsenic-induced toxicity. The results highlight a clear dose-dependent upregulation of stress proteins in response to increasing concentrations of arsenic, which correlates with the induction of oxidative stress and histopathological damage in the gills. The expression of these proteins provides an early warning of arsenic contamination, making them valuable bioindicators for assessing environmental toxicity. The data also underscore the protective role of stress proteins in maintaining cellular integrity under arsenic-induced stress, and the gills of *Clarias batrachus* serve as an ideal site for monitoring arsenic contamination in aquatic ecosystems. Overall, this study contributes to the growing body of knowledge on the use of stress proteins as reliable biomarkers for environmental monitoring and the assessment of toxicological risks in aquatic organisms.

IV. Conclusion

Present study on the role of stress proteins in the gills of *Clarias batrachus* as biomarkers of arsenic-induced toxicity reveals significant insights into the molecular responses of aquatic organisms to environmental contaminants. Arsenic exposure causes a range of toxic effects including oxidative stress and immune dysfunction which are mitigated through the upregulation of stress proteins like heat shock proteins and metallothioneins. These proteins play essential roles in protecting cells from damage and maintaining homeostasis under stress conditions. The use of *Clarias batrachus* as a model organism has demonstrated that the gills, being a primary site of arsenic uptake, serve as an effective organ for monitoring arsenic-induced toxicity. The findings emphasize the potential of stress proteins as reliable biomarkers for assessing arsenic pollution in aquatic ecosystems, contributing to environmental health monitoring and the development of mitigation strategies. By understanding the molecular mechanisms of arsenic toxicity, this research aids in improving the ecological risk assessment of heavy metal contamination and supports efforts to protect aquatic organisms and their habitats from toxic pollutants.

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