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# STRESS PROTEIN DYNAMICS IN THE GILLS OF ARSENIC-EXPOSED *CLARIAS GARIEPINUS*: A BIOMARKER EVALUATION

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## ABSTRACT

Arsenic contamination in aquatic ecosystems presents a significant environmental concern with fish serving as primary indicators of water quality and pollutants. This study aimed to evaluate the expression of stress proteins in the gills of *Clarias gariepinus* (African catfish) exposed to arsenic using these proteins as biomarkers for arsenic-induced oxidative stress. A short-term (96-hour) exposure to varying concentration of arsenic (1 mg/L, 5 mg/L and 10 mg/L) was conducted. Protein expression levels of HSP70 and HSP90 were assessed using SDS-PAGE .The results revealed significant dose dependent increases in the expression of these stress proteins indicating a strong cellular response to oxidative stress. Additionally, biomarkers of oxidative stress such as catalase and superoxide dismutase (SOD) showed enhanced activity in exposed fish reinforcing the notion that arsenic induces oxidative damage. The findings highlighted the utility of stress proteins and enzymes as effective biomarkers for assessing arsenic contamination in aquatic environments and emphasize the need for further research into the molecular mechanisms underlying arsenic toxicity in aquatic species.

**KEYWORDS:** Arsenic, *Clarias gariepinus*, stress proteins, oxidative stress, biomarkers, gills.

## INTRODUCTION

Arsenic contamination of aquatic ecosystems is a critical environmental issue that poses significant risks to aquatic organisms including fish. Arsenic is a naturally occurring element that is widely distributed in the earth's crust and it can enter water bodies through both natural and anthropogenic sources. The primary anthropogenic sources of arsenic

in aquatic environments include industrial discharges, mining activities, the use of pesticides, and agricultural runoff which contribute to the contamination of freshwater ecosystems.[1,2] In regions with high arsenic levels, fish populations can be exposed to toxic concentrations, leading to various physiological, biochemical, and molecular alterations. *Clarias gariepinus*, commonly known as the African catfish, is an important freshwater species that is widely distributed in various aquatic environments across the world. Due to its resilience in laboratory conditions, its significance in aquaculture, and its adaptability to various environmental conditions, *Clarias gariepinus* has been widely used in toxicological studies.[3,4] This species like many other aquatic organisms is vulnerable to the toxic effects of pollutants including arsenic which may induce cellular stress, disrupt biological functions and impact overall health and survival.[5,6]

Arsenic toxicity in aquatic organisms has been a subject of numerous studies particularly focusing on its effects on the physiology, biochemistry, and molecular biology of fish. Exposure to arsenic leads to oxidative stress, a condition in which the production of reactive oxygen species (ROS) overwhelms the body's antioxidant defense mechanisms.[7,8] ROS can cause damage to cellular macromolecules including lipids, proteins and DNA, resulting in a range of harmful effects such as impaired cellular function, inflammation, and increased susceptibility to diseases.[9,10]

In fish, the gills are the primary organ responsible for respiration, osmoregulation, and excretion, making them particularly vulnerable to environmental pollutants. The gills are directly exposed to waterborne contaminants like arsenic and their cells are frequently subjected to oxidative damage caused by the uptake of pollutants. Consequently, the gills of fish can serve as an ideal target for studying the molecular and cellular responses to arsenic exposure as they reflect both the biochemical changes and the damage caused by oxidative stress.[11,12]

Stress proteins particularly heat shock proteins (HSPs) are a group of molecular chaperones that play a critical role in maintaining cellular homeostasis under stress conditions. HSPs help cells cope with stress by assisting in the proper folding of proteins, preventing protein aggregation, and repairing damaged proteins.[13,14] Among the most studied stress proteins are HSP70 and HSP90 which are involved in cellular protein quality control and stress responses. In fish, as in other organisms, the expression of stress proteins is a key indicator of cellular stress and damage caused by environmental pollutants including heavy metals like arsenic. The upregulation of HSPs has been widely recognized as a biomarker for environmental stress in fish and they are often used to assess the impact of pollutants on aquatic ecosystems.[15,16] The expression of stress proteins in response to arsenic exposure has been documented in several fish species but studies on *Clarias gariepinus* are relatively limited. Previous research has shown that arsenic exposure leads to an increase in the expression of HSP70 and HSP90 in various fish species, indicating that these proteins play a vital role in the fish's defense mechanisms against arsenic-induced oxidative stress.[17,18] The upregulation of these stress proteins serves as a protective response to mitigate the cellular damage caused by arsenic, and their expression levels can be used to assess the extent of arsenic toxicity in fish. However the precise molecular mechanisms underlying the induction of stress proteins in *Clarias gariepinus* exposed to arsenic as well as the specific pathways involved, remain poorly understood. This knowledge gap highlights the need for further investigation into the dynamics of stress protein expression in response to arsenic exposure particularly in the gills of *Clarias gariepinus*.[19,20]

The primary objective of this study is to evaluate the expression of stress proteins, specifically HSP70 and HSP90 in the gills of *Clarias gariepinus* exposed to arsenic. This study will explore the dose-dependent effects of arsenic on the expression of these stress proteins and examine how exposure to different concentrations of arsenic affects the

molecular responses of the fish. By focusing on the gills, this research aims to provide a better understanding of the molecular mechanisms involved in arsenic toxicity and to investigate the potential of stress proteins as biomarkers for arsenic contamination in aquatic ecosystems.[21,22] The gills are an essential organ in fish, responsible for gas exchange, ion regulation, and excretion. As the first line of defense against waterborne pollutants, the gills are particularly vulnerable to oxidative damage caused by pollutants like arsenic, making them a suitable tissue for assessing the effects of arsenic exposure at the molecular level.[23,24]

In addition to stress proteins, this study will also assess the activities of other biomarkers of oxidative stress, such as catalase and superoxide dismutase (SOD), in the gills of *Clarias gariepinus*. These enzymes play a crucial role in protecting cells from oxidative damage by neutralizing ROS and maintaining the balance between pro-oxidants and antioxidants. The activities of catalase and SOD will be measured to provide additional insights into the oxidative stress response induced by arsenic exposure. Moreover, this study will explore the potential for combining stress proteins with other biomarkers of oxidative stress to develop a more comprehensive and sensitive approach for monitoring arsenic contamination in aquatic ecosystems.[25,26]

The significance of this study lies in its potential to contribute to the growing body of knowledge on arsenic toxicity in fish and its molecular effects on aquatic organisms. Arsenic contamination is a global issue that affects freshwater ecosystems, and understanding how arsenic impacts fish at the molecular level is essential for developing strategies to mitigate its harmful effects. By evaluating stress protein expression in response to arsenic exposure, this study aims to provide a valuable tool for monitoring environmental contamination and assessing the health of fish populations. The findings of this study could also inform regulatory policies regarding arsenic levels in water bodies, leading to improved water quality management and the protection of aquatic biodiversity.[27,28]

Previous researches have explored the effects of other heavy metals on fish; there is limited information available on how arsenic exposure specifically affects the molecular and biochemical responses of *Clarias gariepinus*. This research will address this gap by providing detailed molecular data on the induction of stress proteins in response to arsenic exposure and examining the relationship between arsenic-induced oxidative stress and cellular damage in fish

Overall, this research will contribute to the understanding of arsenic toxicity in fish, focusing on the expression of stress proteins as biomarkers of environmental contamination. By examining the molecular responses of *Clarias gariepinus* exposed to arsenic, the study will provide insights into the ecological risks of arsenic pollution in aquatic ecosystems and the role of stress proteins in mitigating the toxic effects of arsenic. The findings of this study could be used to develop more effective monitoring tools for assessing arsenic contamination in freshwater environments and help inform policies aimed at protecting aquatic organisms from the harmful effects of arsenic exposure. This study will also pave the way for future research on the molecular mechanisms of arsenic toxicity in fish and other aquatic organisms, further enhancing our understanding of the impact of arsenic pollution on aquatic biodiversity.[1,17,19]

## METHODOLOGY

The experiment was designed to see the effects of arsenic on stress proteins and oxidative enzymes in the gills of *Clarias gariepinus* following exposure to varying concentration of arsenic for 96 hours. The study utilized healthy, mature male *C. gariepinus* which were maintained in controlled laboratory conditions for one week for the acclimation. After acclimation, fish were exposed to varying concentrations of arsenic (0 mg/L, 1 mg/L, 5 mg/L, and 10 mg/L) for

96 hours and the gills were dissected out and processed for the analysis of stress proteins, catalase and superoxide dismutase (SOD) using the standard protocols. The protein extraction process followed the established protocols ensuring that proteins are efficiently solubilized for subsequent analysis.

Statistical analysis including One Way ANOVA followed by Tukey post hoc test was performed to determine significant differences in stress protein expression and oxidative stress marker activities between exposed and control groups.

## RESULTS AND DISCUSSION

The study revealed the effects of arsenic exposure on *Clarias gariepinus* through acute and chronic toxicity experiments focusing on stress protein expression (HSP70 and HSP90) and oxidative stress markers (catalase and SOD activities). The data showed significant increase in stress proteins and oxidative stress activities in the gills of the fish exposed to higher arsenic concentrations.

The SDS-PAGE analysis revealed a clear dose-dependent upregulation of HSP70 and HSP90 in the gill tissues of *Clarias gariepinus* exposed to arsenic for 96 hours. The optical density (OD) of protein bands was quantified using densitometry and normalized against the control.

**Table 1: Table 1 shows mean band intensity of HSP70 and HSP90 after exposure to varying concentration of arsenic for 96 hours. Note a significant ($p < 0.05$) increases in both the proteins were observed at 5 mg/L and 10 mg/L.**

| Arsenic Concentration (mg/L) | HSP70 Band Intensity (Mean ± SD) | HSP90 Band Intensity (Mean ± SD) |
|---|---|---|
| 0 mg/L (Control) | 0.35 ± 0.02 | 0.30 ± 0.01 |
| 1 mg/L | 0.45 ± 0.03 | 0.40 ± 0.02 |
| 5 mg/L | 0.60 ± 0.04 | 0.55 ± 0.03 |
| 10 mg/L | 0.75 ± 0.05 | 0.70 ± 0.04 |

Stress proteins are the molecular chaperones that assist in maintaining cellular homeostasis under stress conditions by facilitating the proper folding of proteins, preventing protein aggregation and repairing damaged proteins.[9] HSP70 and HSP90 are two well-known stress proteins involved in the cellular response to environmental stress.[10] HSP70 is a major heat shock protein that is involved in protein folding and protecting cells from heat shock and other stresses including those induced by pollutants like arsenic.[11] Similarly, HSP90 plays a crucial role in maintaining cellular protein integrity under stress conditions and is involved in various cellular processes, including signal transduction, cellular trafficking, and the regulation of protein degradation.[12] These proteins are highly conserved across species, making them valuable biomarkers for studying the effects of pollutants on aquatic organisms.

The upregulation of these proteins serves as a molecular indicator of oxidative stress and cellular damage caused by environmental contaminants. The induction of HSPs is considered an adaptive response to mitigate the damage caused by oxidative stress and maintain cellular integrity. As a result, stress proteins particularly HSP70 and HSP90 have been widely recognized as useful biomarkers for monitoring the impact of arsenic and other heavy metals on aquatic organisms.[15,16]

### Oxidative Stress Biomarker Activity

The results indicate that arsenic exposure elevates ROS levels, stimulating the antioxidant defense system.

**Table 2: Shows the activity of catalase enzyme (U/mg protein ± SD) in gill after exposure to varying concentration of arsenic for 96 hours.**

| Arsenic Concentration (mg/L) | Catalase Activity (U/mg protein ± SD) |
|---|---|
| 0 (Control) | 15.2 ± 1.1 |
| 1 mg/L | 18.5 ± 1.3 |
| 5 mg/L | 23.7 ± 1.6 |
| 10 mg/L | 29.1 ± 1.8 |

**Table 3: Shows the activity of SOD enzyme (U/mg protein ± SD) in gill after exposure to varying concentration of arsenic for 96 hours.**

| Arsenic Concentration (mg/L) | SOD Activity (U/mg protein ± SD) |
|---|---|
| 0 (Control) | 22.6 ± 1.4 |
| 1 mg/L | 27.4 ± 1.6 |
| 5 mg/L | 32.9 ± 1.9 |
| 10 mg/L | 38.5 ± 2.0 |

Both enzymes showed a positive correlation ($r > 0.9$) with arsenic concentration. The data confirm that arsenic induces oxidative stress which is countered by upregulation of antioxidant enzymes.

Studies have demonstrated that arsenic exposure leads to an increase in ROS production, which, in turn, causes lipid peroxidation, protein oxidation, and DNA damage in fish tissues.[17,18] In the gills of fish, the increased production of ROS due to arsenic exposure can disrupt the cellular integrity of the epithelial cells, leading to changes in gill morphology and function.[19] The gills, being directly exposed to waterborne pollutants, are highly susceptible to arsenic-induced oxidative damage, which can impair the fish's ability to regulate ion balance, oxygen uptake and waste elimination.[20]

## CONCLUSION

This study provides valuable insights into the effects of arsenic exposure on *Clarias gariepinus* with a particular focus on the expression of stress proteins in the gills of the fish. The results indicate that short term arsenic exposure induces a dose-dependent upregulation of key stress proteins such as HSP70 and HSP90 which play a critical role in the fish’s defense mechanisms against oxidative stress. This upregulation of stress proteins serves as a protective cellular response aimed at mitigating the damage caused by arsenic-induced oxidative stress.

The findings of this study suggest that arsenic exposure leads to significant oxidative stress in *Clarias gariepinus* as evidenced by the increased activity of catalase and superoxide dismutase (SOD) enzymes. These enzymes are part of the antioxidant defense system that helps to neutralize the reactive oxygen species (ROS) generated by arsenic exposure. The increased activities of catalase and SOD in arsenic-exposed fish further emphasize the cellular response to oxidative damage, indicating the stress experienced by the fish at the molecular level.

In conclusion this study provides a comprehensive understanding of the biochemical, effects of arsenic exposure on *Clarias gariepinus*. The findings emphasize the need for continued research into the long-term effects of arsenic exposure on aquatic organisms and the development of more effective strategies for mitigating the impacts of environmental pollution on aquatic ecosystems. Future research should explore additional biomarkers and stress responses in various fish species to further expand our understanding of arsenic toxicity and its ecological consequences.

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