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Effects of benzalkonium chloride exposure on physiological and behavioural responses in freshwater fish *Catla catla*

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Abstract
The extensive use of domestic and industrial chemicals has contributed to noticeable environmental alterations. The present study investigates the xenobiotic effects of benzalkonium chloride on the gill and muscle tissues of the freshwater fish *Catla catla*. Fingerlings of *Catla catla* (average weight: 5.2 ± 1.3 g; average length: 6.0 ± 1.8 cm) were obtained from the Turvekere fishers farm in Tumkur, Karnataka. Acute toxicity was evaluated using static bioassay experiments. Groups of ten well-acclimatized fish were exposed to different concentrations of benzalkonium chloride to determine the 24-hour LC_{50} values. Mortality and behavioral responses were observed at two-hour intervals. Biochemical parameters such as glucose, glycogen, and protein levels were analyzed to assess physiological alterations. Fish exposed to sublethal concentrations of benzalkonium chloride showed a marked decrease in glycogen content in both gill and muscle tissues compared to the control group. Protein levels in these tissues were also reduced. In contrast, glucose levels were elevated in both tissues. The depletion of glycogen may be linked to stress-induced increases in metabolic demand, while the reduction in protein levels could result from the breakdown of amino acids to meet energy requirements under toxic stress. This study provides new insights into the toxic effects of benzalkonium chloride on *Catla catla*, especially in juvenile stages, which are more sensitive to chemical pollutants than adult fish.

Keywords: *Catla catla*, benzalkonium chloride, desquamation, hyperglycemia

Introduction

Background

The extensive use of household and industrial chemicals in modern society has significantly contributed to environmental pollution, particularly in aquatic ecosystems. Among these substances, benzalkonium chloride (BAC), a quaternary ammonium compound widely used in disinfectants and cleaning products, is valued for its strong antimicrobial and surface-active properties. Due to its high effectiveness in eliminating bacteria, fungi, and viruses, benzalkonium chloride is extensively applied in domestic sanitation, healthcare settings, and industrial cleaning processes. However, improper disposal and discharge of wastewater containing BAC can lead to its accumulation in natural water bodies, posing risks to aquatic organisms (Hughes and Endicott, 2010; Chakraborty and Bhowmik, 2019) [5, 7]. Surfactants such as benzalkonium chloride function by reducing the surface tension of water, thereby enhancing the removal of dirt and microbial contaminants. Despite these benefits, their release into aquatic environments can disturb physicochemical properties of water, alter membrane permeability in aquatic organisms, and interfere with respiratory and metabolic processes in fish and another biota (Sharma and Sharma, 2019) [15]. Quaternary ammonium compounds are known to adsorb onto biological membranes, potentially causing gill damage, oxidative stress, and enzyme dysfunction, which may ultimately affect growth, reproduction, and survival rates of aquatic species (Ali and Khan, 2020) [2]. The impact of such contaminants on fish has drawn increasing attention because fish serve as important bioindicators of aquatic pollution. Exposure to sublethal concentrations of surfactants can lead to behavioural stress responses, impaired swimming activity, and biochemical alterations such as changes in carbohydrate, protein, and lipid metabolism (Sree and Suresh, 2022) [18]. Moreover, early life stages of fish are particularly vulnerable, as their detoxification systems are not fully developed, making them more susceptible to xenobiotic toxicity. Continuous input of these chemicals into freshwater systems may therefore contribute to long-term ecological imbalance and biodiversity loss.

Importance of Studying Fish

Fish are key indicators of the health of aquatic environments and are commonly used in bioassays to determine the toxicity of environmental pollutants (Ali and Khan, 2020; Singh and Dhillon, 2017) [2, 16]. Their physiological and biochemical responses to pollutants provide valuable information about the overall effects of these contaminants on aquatic ecosystems. Among the different tissues and organs in fish, the gills and muscles are particularly prone to be affected by toxic substances, as they come into direct contact with water and are crucial for respiration and movement (Gupta and Kumar, 2019; Kaur and Kumar, 2020) [6, 8].

Benzalkonium chloride and Its Components

Benzalkonium chloride contains a mixture of surfactants, phenolic compounds, and other chemical agents designed to clean and disinfect. While effective in its intended use, the discharge of Benzalkonium chloride into water bodies poses a risk of contamination. The surfactants and phenolic compounds can interfere with the physiological processes in fish, potentially leading to adverse effects such as oxidative

stress, disruption of osmotic balance, and tissue damage (Saini and Kumar, 2021; Biswas and Mandal, 2021; Adhikari and Rana, 2018) [1, 4, 13].

Objectives of the Study

The primary objective of this study is to evaluate the acute toxicity of Benzalkonium chloride on freshwater fish *Catla catla* and to assess the biochemical and behavioural changes induced by Benzalkonium chloride exposure. Specifically, this study aims

- **Identify LC_{50} Values:** Determine the concentration of Benzalkonium chloride that results in a 50% mortality rate (LC_{50}) in *Catla catla*, through systematic exposure trials.
- **Examine Biochemical Alterations:** Investigate the variations in glucose, glycogen, and protein concentrations within the gill and muscle tissues of *Catla catla*, subjected to different levels of Benzalkonium chloride.
- **Analyze Behavioral Changes:** Monitor and record behavioral changes in *Catla catla*, exposed to varying concentrations of Benzalkonium chloride, and establish a connection between these behavioral changes and the biochemical data obtained.

Significance of the Study

Understanding the effects of Benzalkonium chloride on freshwater fish is important for multiple reasons. It allows for evaluating the environmental risks associated with household and industrial cleaning products. It also sheds light on the mechanisms of toxic action in aquatic organisms, which can help shape regulatory practices and environmental protection strategies (Chakraborty and Banerjee, 2022; Saini and Kumar, 2021) [13]. Additionally, this study adds to the broader field of aquatic toxicology, supporting the development of safer cleaning products and improved waste disposal methods (Singh and Dhillon, 2017; Adhikari and Rana, 2018) [1, 16].

Material and Methods

1. Selection of Test Organisms

Fingerlings of *Catla catla* (weight: 5.2±1.3 g; length: 6.0±1.8 cm) were collected from the Turvekere fishers farm Tumkur, Karnataka. And transported to the laboratory in well-ventilated polyethylene bags to prevent injury. The test organisms were housed in large plastic containers and acclimatized to laboratory conditions.

2. Bioassay Protocol

The study will be conducted under the OECD guideline No. 203 in static test conditions (OECD, 1992) and the experiment will be set in duplicate to obtain the 24-hour LC_{50} value of the test chemical for the species. Static bioassay tests were conducted to evaluate the acute toxicity of Benzalkonium chloride. Dechlorinated tap water was used for acclimation as well as for the experiment and control. In each treatment, ten fully acclimatized fish of each species were exposed to varying concentrations of Benzalkonium chloride to determine LC_{50} values after 24 hours. The fish were fed twice daily with groundnut oil cake and rice bran, amounting to 10% of their body weight. The moderate-sized fish were selected for the experiment and exposed to different concentrations of Benzalkonium chloride.

3. LC_{50}- Determination:

Commercially available Benzalkonium chloride was used, with concentrations measured in ml/L. Four troughs, each containing 10 litres of water, were stocked with 10 fish species and exposed to different concentrations of Benzalkonium chloride (0.01, 0.02, 0.03, 0.04, 0.05, and 0.06 ml/L). A control set, with the same number of fish and water volume but without Benzalkonium chloride, was maintained. The experiment was conducted in duplicate. The water was aerated, and feeding was halted during the experiment. Dead fish were removed and counted at 24 hours, and LC_{50} values were calculated using the Tabular method.

4. Study of Behavioural Response

Behavioural changes in the fish were observed immediately after the application of the test substance and throughout the experiment. The control group exhibited normal behaviour throughout the experiment. At the lowest concentration (0.01 ml/L), the fish showed normal responses. However, as the concentration increased, notable changes in behaviour were observed, including erratic swimming patterns, loss of balance, and rapid opercular movements. At the highest concentration (0.06 ml/L), the severity of these responses increased, with fish showing severe loss of balance, lying laterally at the bottom, and rapid opercular movements with an open mouth.

5. Biochemical Analysis

At the end of each exposure period, the fish were sacrificed, and tissues such as gills and muscles were dissected for biochemical analysis. Glucose and glycogen were estimated by using Kemp's method (Kemp and Heijningen, 1954) [9] and protein content by the Lowry method (Lowry *et. al.*,1951) [11].

Results

1. Toxicity Studies

Lethal concentration for 50% of the test organisms (LC_{50}) is the concentration at which 50% of the experimental animals survive. LC_{50} values are determined by plotting the concentration on the X-axis and the percentage of survival on the Y-axis. A straight line is drawn between the maximum points representing survival at various concentrations, and the LC_{50} is identified as the concentration at which this line crosses the 50% survival mark.

The LC_{50} value of Benzalkonium chloride for *Catla catla* fingerlings was determined to be 0.05 ml/L.

Table 1: LC_{50} Values of Benzalkonium chloride Exposure to Fingerlings of *Catla catla*

Cleaner	Fish Species	Exposure Period	Method	LC50
Benzalkonium chloride	*Catla catla*	24 hours	Tabular	0.05 ml/L

There was no mortality in the control group at the end of the experiment (24 hours). At the highest concentrations (0.1 ml/L and 0.06 ml/L) of Benzalkonium chloride, mortality was 100% for 0.1 ml/L for *Catla catla*. The estimated 24-hour LC_{50} values for Benzalkonium chloride in *Catla catla* fingerlings using a static bioassay system were 0.05 ml/L.

2. Behavioural Studies

Behavioural responses of *Catla catla* to different concentrations of Benzalkonium chloride were observed throughout the experimental period. The control group exhibited normal behaviour throughout the experiment. At the lowest concentration (0.01 ml/L), the fish showed normal responses. However, as the concentration increased, notable changes in behaviour were observed in both species, including erratic swimming, occasional gasping for breath, loss of balance, and spiralling movements with jerks. At the highest concentration, 0.05 ml/L for *Catla catla* the severity of these responses increased, with fish showing severe loss of balance, lying laterally at the bottom, spiralling movements with jerks, and rapid opercular movements with an open mouth.

Table 2: Behavioural Responses of *Catla catla* Fingerlings During Exposure to Lethal Concentrations of Benzalkonium chloride

Behavior	0.01 ml/L	0.02 ml/L	0.03 ml/L	0.04 ml/L	0.05 ml/L	0.06 ml/L
Normal swimming	+	+	_	_	_	_
Erratic swimming and gasping	_	+	+	+	+	+
Loss of balance	_	_	+	+	+	+
Spiralling movements with jerks	_	_	_	+	+	+
Lying laterally at the bottom	_	_	_	_	+	+
Rapid opercular movements with open mouth	_	_	_	_	_	+

3. Biochemical Studies

3.1 Glucose: Exposure to Benzalkonium chloride increased glucose levels in fish tissues. In *Catla catla*, glucose content in muscles and gills was 0.142 mg/g and 0.091 mg/g, respectively, compared to 0.179 mg/g and 0.115 mg/g in Benzalkonium chloride-exposed fish.

Table 3: Glucose Content in Tissues of *Catla catla* Exposed to Benzalkonium chloride

Tissue	Species	Control	Benzalkonium chloride Exposed
Muscle	*Catla catla*	0.142 mg/g	0.179 mg/g
Gills	*Catla catla*	0.091 mg/g	0.115 mg/g

3.2 Glycogen: Glycogen content decreased significantly in Benzalkonium chloride-exposed fish tissues. For *Catla catla*, glycogen content in muscles and gills was 0.020 mg/g and 0.065 mg/g, respectively, compared to 0.130 mg/g and 0.125 mg/g in control fish.

Table 4: Glycogen Content in Tissues of *Catla catla* Exposed to Benzalkonium chloride

Tissue	Species	Control	Benzalkonium chloride Exposed
Muscle	*Catla catla*	0.130 mg/g	0.020 mg/g
Gills	*Catla catla*	0.125 mg/g	0.065 mg/g

3.3 Protein: Protein levels were significantly reduced in Benzalkonium chloride-exposed fish. For *Catla catla*, protein content in muscles and gills was 0.490 mg/g and 0.760 mg/g, respectively, compared to 0.660 mg/g and 0.880 mg/g in control fish.

Table 5: Protein Content in Tissues of *Catla catla* Exposed to Benzalkonium chloride

Tissue	Species	Control	Benzalkonium chloride Exposed
Muscle	*Catla catla*	0.660 mg/g	0.490 mg/g
Gills	*Catla catla*	0.880 mg/g	0.760 mg/g

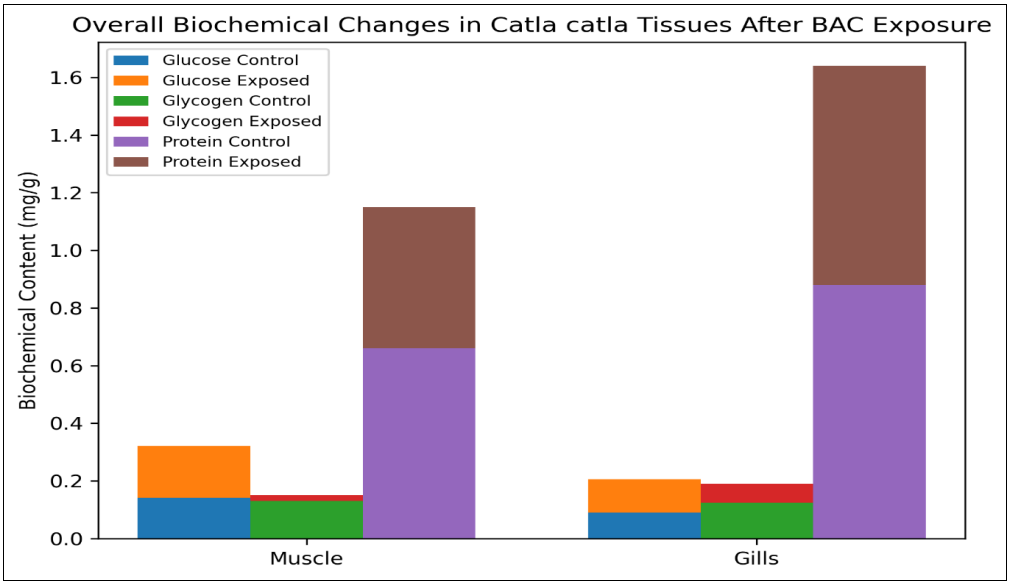

Fig 1: Graph Representing the Biochemical Changes in *Catla catla*

Discussion

This study elucidates the acute toxicity and sub-lethal effects of Benzalkonium chloride, a commonly used floor cleaner, on freshwater fish species *Catla catla*. The findings highlight the severe impact of Benzalkonium chloride exposure on the physiological and biochemical integrity of

these aquatic organisms, as evidenced by significant alterations in both biochemical markers.

1. Biochemical Impacts

1.1 Glucose Levels

The study observed a marked increase in glucose levels in both muscle and gill tissues of *Catla catla* exposed to Benzalkonium chloride. Specifically, the glucose content in muscle tissues increased from 0.142 mg/g in control fish to 0.179 mg/g in Benzalkonium chloride-exposed fish, while in gill tissues, it increased from 0.091 mg/g to 0.115 mg/g. These elevated glucose levels suggest that Benzalkonium chloride exposure induces significant stress, leading to hyperglycemia. This response is likely due to the activation of stress mechanisms that mobilize energy reserves, particularly glucose, to cope with the toxic insult. The observed hyperglycemia is consistent with findings from other studies that report similar biochemical responses in fish subjected to toxic substances (Sastry and Rao, 2004; Kumar and Sharma, 2015) [10, 14, 15].

1.2 Glycogen Levels

Conversely, glycogen levels were significantly reduced in the tissues of *Catla catla* exposed to Benzalkonium chloride. In muscle tissues, glycogen content decreased from 0.130 mg/g in control fish to 0.020 mg/g in exposed fish, and in gill tissues, it dropped from 0.125 mg/g to 0.065 mg/g. This glycogen depletion indicates that Benzalkonium chloride-induced stress leads to the exhaustion of energy reserves, as the fish utilize stored glycogen to meet the heightened energy demands during stress. The reduction in glycogen levels reflects impaired metabolic processes and energy imbalances caused by toxic exposure (Ali and Dutta, 2001) [3].

1.3 Protein Levels

In *Catla catla* exposed to Benzalkonium chloride, protein levels were significantly reduced. Specifically, the protein content in muscle tissues decreased from 0.660 mg/g in control fish to 0.490 mg/g in Benzalkonium chloride-exposed fish, and in gill tissues, it dropped from 0.880 mg/g to 0.760 mg/g. This reduction in protein levels highlights the metabolic disturbances induced by Benzalkonium chloride. Proteins are essential for maintaining structural integrity and various physiological functions, and their decrease may result from increased proteolysis and the use of proteins as an alternative energy source under stress. This finding is consistent with observations of protein depletion in fish exposed to various pollutants (Singh and Gupta, 2014; Yadav and Kumar, 2018) [17, 19].

2. Behavioural Changes

As Benzalkonium chloride concentration increased, *Catla catla* exhibited various behavioral changes. At 0.01 ml/L and 0.02 ml/L, the fish displayed normal swimming, which was not observed at higher concentrations. Erratic swimming and gasping began at 0.02 ml/L and became more pronounced with increasing concentrations, occurring at all higher levels. Loss of balance was evident starting at 0.03 ml/L and continued at greater concentrations. Spiraling movements with jerks were first seen at 0.04 ml/L and persisted at higher levels. Lying laterally at the bottom was observed from 0.05 ml/L, and rapid opercular movements with an open mouth were noted only at the highest concentration of 0.06 ml/L.

These behavioral alterations, including erratic swimming and increased opercular movements, reflect stress and discomfort caused by Benzalkonium chloride exposure. Such behaviors often signal compromised respiratory function and overall distress, supporting the biochemical and histological findings reported in similar studies (Rajput and Choudhury, 2017) [12].

Conclusion

This study provides a comprehensive assessment of the effects of Benzalkonium chloride on *Catla catla.* The acute toxicity tests reveal that Benzalkonium chloride has a pronounced impact on fish health, with LC_{50} values indicating significant risks at relatively low concentrations. The biochemical assays demonstrate that Benzalkonium chloride exposure leads to increased glucose levels, decreased glycogen, and reduced protein levels, reflecting substantial metabolic stress and disturbances.

Recommendations for Future Research

1. **Long-Term Studies:** Conduct chronic exposure studies to evaluate the long-term impacts of Benzalkonium chloride on fish health and reproduction.
2. **Multiple Species Analysis:** Investigate the effects of Benzalkonium chloride on other freshwater and marine species to assess its broader ecological impact.
3. **Mechanistic Studies:** Explore the molecular mechanisms underlying Benzalkonium chloride-induced stress and tissue damage to develop targeted mitigation strategies.
4. **Environmental Monitoring:** Implement regular monitoring of aquatic environments for the presence of household and industrial chemicals to prevent contamination and protect aquatic ecosystems.

This research underscores the importance of addressing the environmental impact of household chemicals like Benzalkonium chloride and developing effective strategies to mitigate their effects on aquatic life.

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