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Toxicological assessment of Benzalkonium Chloride on behavioral and histological parameters in *Catla Catla*

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Abstract
The increasing use of household and industrial chemicals has led to considerable environmental disturbances, particularly affecting aquatic ecosystems. This study evaluates the xenobiotic effects of Benzalkonium Chloride on the freshwater fish *Catla catla*, with special emphasis on behavioral, biochemical, and histological alterations in gill and muscle tissues. Fingerlings of *Catla catla* (average weight 5.2 ± 1.3 g; length 6.0 ± 1.8 cm) were procured from a fish farm in Tumkur, Karnataka, and subjected to static bioassay tests to determine acute toxicity. Groups of acclimatized fish were exposed to varying concentrations of Benzalkonium Chloride to estimate the 24-hour LC_{50}, while behavioral responses and mortality were monitored at regular 2-hour intervals. Biochemical analyses revealed a significant decline in glycogen and protein levels in both gill and muscle tissues compared to controls, accompanied by an elevation in glucose levels, indicating stress-induced metabolic disturbances and increased energy demand. The depletion of glycogen may be linked to enhanced metabolic activity, whereas reduced protein content suggests the utilization of amino acids in stress-related catabolic processes. Histopathological observations demonstrated severe damage to gill tissues, including epithelial desquamation, necrosis (telangiectasia), shrinkage of secondary lamellae, and hypertrophy and hyperplasia at the base of lamellae. Muscle tissues showed nuclear proliferation, variation in fiber size, disintegration and atrophy of muscle bundles, along with thickening and separation of fibers. Overall, the findings highlight the toxic impact of Benzalkonium Chloride on *Catla catla*, particularly emphasizing the susceptibility of early developmental stages to chemical exposure.

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

Introduction

In recent years, the extensive use of household and industrial chemicals has significantly contributed to environmental pollution, particularly in aquatic ecosystems. Among these, surfactant-based compounds such as Benzalkonium Chloride are widely used for their strong disinfectant and antimicrobial properties. Benzalkonium Chloride, a common quaternary ammonium compound present in many cleaning and sanitizing products, can enter water bodies through improper disposal and runoff, posing potential risks to aquatic organisms. Surfactants are known to reduce the surface tension of water, thereby enhancing the solubility and dispersion of various substances; however, their presence in aquatic environments can disrupt the natural physicochemical balance and adversely affect aquatic life. These compounds can interfere with cellular membranes, respiration, and metabolic processes in fish. Increasing evidence indicates that exposure to such xenobiotics may lead to toxicological, physiological, and histopathological alterations, particularly in sensitive species like *Catla catla*, thereby threatening aquatic biodiversity and ecosystem stability (Kumar and Singh, 2021; Sharma and Kaur, 2020; Yadav *et al*., 2022; Verma *et al.,* 2023) [5, 8, 9, 10]. Hence, evaluating the impact of Benzalkonium Chloride on *Catla catla* has become essential for understanding its ecological consequences and ensuring the protection of freshwater environments.

Importance of studying fish

Fish serve as vital bioindicators for assessing the health of aquatic ecosystems and are widely employed in toxicological studies to evaluate the impact of environmental pollutants. Their physiological and biochemical responses offer crucial insights into the effects of contaminants at both organismal and ecosystem levels. In particular, tissues such as gills and muscles are highly sensitive to toxic exposure. Gills are directly exposed to the aquatic environment and play a key role in respiration and ion regulation, making them especially vulnerable to chemical pollutants. Muscle tissues, on the other hand, are essential for locomotion and energy storage and can reflect metabolic disturbances caused by toxicants. Therefore, studying these tissues in *Catla catla* provides valuable information on pollutant-induced stress and toxicity (Kumar and Singh, 2021; Yadav *et al.,* 2022) [5, 10].

Benzalkonium chloride and its components

Benzalkonium Chloride is a widely used quaternary ammonium compound present in disinfectants, sanitizers, and cleaning agents due to its strong antimicrobial properties. Despite its effectiveness, its release into aquatic environments raises serious ecological concerns. Benzalkonium Chloride acts as a cationic surfactant that can disrupt cell membranes and interfere with normal physiological processes in aquatic organisms. Studies have reported that exposure to such compounds can induce oxidative stress, alter enzyme activity, disturb osmotic balance, and lead to tissue damage in fish. The accumulation of these chemicals in water bodies can therefore pose significant risks to species like *Catla catla*, affecting their survival and overall health (Verma *et al.,* 2023; Zhang *et al.,* 2022) [9, 11].

Objectives of the study

The primary objective of the present investigation is to evaluate the acute toxicity of Benzalkonium Chloride on the freshwater fish *Catla catla* and to analyze the associated biochemical and histological alterations. The specific objectives include:

1. **Determination of LC_{50} Values:** To estimate the median lethal concentration (LC_{50}) of Benzalkonium Chloride for *Catla catla*.

2. **Assessment of Biochemical Changes:** To evaluate variations in glucose, glycogen, and protein levels in the gill and muscle tissues of exposed fish.

3. **Examination of Histopathological Effects:** To study structural alterations in gill and muscle tissues in response to Benzalkonium Chloride exposure.

4. **Evaluation of Behavioral Responses:** To observe and document behavioral changes in fish under different concentrations of the toxicant and correlate them with biochemical and histological findings.

Significance of the study

Understanding the toxic effects of Benzalkonium Chloride on freshwater fish like *Catla catla* is essential for assessing the ecological risks associated with the widespread use of disinfectants and cleaning agents. This study contributes to identifying the mechanisms of toxicity at biochemical and tissue levels, which is crucial for environmental monitoring and risk assessment. Furthermore, the findings can support the formulation of environmental regulations and promote the development of safer chemical alternatives. It also adds valuable knowledge to the field of aquatic toxicology, aiding in the conservation of aquatic biodiversity and the sustainable management of water resources (Sharma and Kaur, 2020; Verma *et al.,* 2023) [8, 9].

Materials and methods

Selection of test organisms

Fingerlings of *Catla catla* (average weight: 5.2 ± 1.3 g; length: 6.0 ± 1.8 cm) were procured from a fish farm in Tumkur, Karnataka. The fish were carefully transported to the laboratory in well-aerated polyethylene bags to minimize stress and physical injury. Upon arrival, the test organisms were transferred to large plastic containers containing dechlorinated tap water and allowed to acclimatize to laboratory conditions for a suitable period prior to experimentation (OECD, 2021) [7].

Bioassay protocol

Acute toxicity of Benzalkonium Chloride was assessed using a static bioassay method. Dechlorinated tap water was used for both acclimatization and experimental setups. Groups of ten healthy and acclimatized *Catla catla* fingerlings were exposed to different concentrations of Benzalkonium Chloride to determine the median lethal concentration (LC_{50}) over a 24-hour period. The fish were fed twice daily with a mixture of groundnut oil cake and rice bran at approximately 10% of their body weight during acclimatization, but feeding was discontinued during the exposure period. Only moderately sized and active fish were selected for the experiment to ensure uniformity (APHA, 2017; OECD, 2021) [1, 7].

Determination of LC_{50}

Commercial-grade Benzalkonium Chloride was used as the test toxicant, and concentrations were prepared in ml/L. Experimental setups consisted of multiple troughs, each containing 10 liters of water, with ten fish introduced into each trough. The fish were exposed to a series of concentrations (0.01, 0.02, 0.03, 0.04, 0.05, and 0.06 ml/L), along with a control group maintained under identical conditions without the toxicant. All experiments were conducted in duplicate to ensure reliability. Continuous aeration was provided throughout the exposure period, and no feeding was carried out. Mortality was recorded after 24 hours, and LC_{50} values were calculated using standard tabulation methods (Finney, 1971; OECD, 2021) [3, 7].

Study of behavioral responses

Behavioral responses of *Catla catla* were closely monitored immediately after exposure to Benzalkonium Chloride and throughout the experimental duration. Fish in the control group exhibited normal swimming behavior and physiological activity. At lower concentrations (0.01 ml/L), no significant behavioral deviations were observed. However, with increasing concentrations, fish displayed noticeable stress responses, including erratic swimming, loss of equilibrium, and increased opercular movements. At higher concentrations (0.06 ml/L), the severity of behavioral abnormalities intensified, with fish showing pronounced loss of balance, lateral resting at the bottom, and rapid opercular movements accompanied by an open mouth. These behavioral alterations indicate respiratory distress and toxic stress induced by Benzalkonium Chloride exposure (Kumar and Singh, 2021; Zhang *et al.,* 2022) [5, 11].

Biochemical analysis

At the end of each exposure period to Benzalkonium Chloride, experimental fish (*Catla catla*) were carefully sacrificed, and vital tissues such as gills and muscle were excised immediately for biochemical estimations. The collected tissues were rinsed with ice-cold physiological saline to remove blood and debris, blotted dry, and homogenized in appropriate buffer solutions under cold conditions to prevent enzymatic degradation. The homogenates were centrifuged, and the supernatants were used for analysis. Glucose content was determined using the Anthrone method, glycogen levels were estimated following Kemp's method, and total protein content was quantified by the Lowry method. These biochemical parameters were selected as key indicators of metabolic stress and energy utilization under toxicant exposure. Alterations in these biomolecules provide insight into the physiological disturbances caused by Benzalkonium Chloride in *Catla catla* (Lowry *et al.,* 1951; Kemp *et al.,* 1954; Yadav *et al.,* 2022) [4, 6, 10].

Histological analysis

For histopathological examination, *Catla catla* specimens were sacrificed at the end of the exposure period, and gill and muscle tissues were promptly dissected to avoid post-mortem changes. The tissues were fixed in Bouin's fixative for 24–48 hours to preserve cellular architecture, followed by thorough washing in 70% ethanol to remove excess fixative. Dehydration was carried out through a graded series of ethanol (70%, 80%, 90%, and absolute alcohol), after which the tissues were cleared in xylene and embedded

in molten paraffin wax at 56–58°C. Paraffin blocks were sectioned into thin slices of 4–6 µm thickness using a microtome. The sections were then mounted on glass slides, deparaffinized, and stained with hematoxylin and eosin (H&E) for microscopic examination. Histological observations focused on identifying structural alterations such as epithelial damage, necrosis, hypertrophy, hyperplasia, and muscle fiber degeneration. These changes were analyzed to assess the extent of tissue damage induced by Benzalkonium Chloride exposure (Bancroft and Gamble, 2008; Sharma and Kaur, 2020) [2, 8].

Results

Toxicity studies

The median lethal concentration (LC_{50}) represents the concentration of a toxicant at which 50% of the test organisms survive within a specified exposure period. LC_{50} values were determined by plotting the concentration of Benzalkonium Chloride on the X-axis against the percentage survival of *Catla catla* fingerlings on the Y-axis. A best-fit line was drawn to represent survival trends across concentrations, and the LC_{50} value was obtained at the point where the line intersected the 50% survival level.

The 24-hour LC_{50} value of Benzalkonium Chloride for *Catla catla* fingerlings was found to be 0.05 ml/L using the tabular method.

Table 1: LC50 value of benzalkonium chloride exposure to fingerlings of *Catla catla*

Toxicant	Fish Species	Exposure Period	Method	LC_{50}
Benzalkonium Chloride	*Catla catla*	24 hours	Tabular	0.05 ml/L

No mortality was observed in the control group throughout the 24-hour experimental period, indicating suitable experimental conditions. However, mortality increased progressively with increasing concentrations of Benzalkonium Chloride. At the highest concentration tested (0.06 ml/L), 100% mortality was recorded in *Catla catla* fingerlings. Based on the observed mortality data under static bioassay conditions, the 24-hour LC_{50} value for Benzalkonium Chloride was confirmed as 0.05 ml/L, demonstrating its toxic effect on the test species.

Behavioral studies

Behavioral responses of *Catla catla* fingerlings exposed to varying concentrations of Benzalkonium Chloride were continuously monitored during the experimental period. The control group maintained normal swimming activity, equilibrium, and feeding behavior throughout the study. At the lowest concentration (0.01 ml/L), fish exhibited no noticeable deviation from normal behavior. However, with increasing concentrations of Benzalkonium Chloride, progressive behavioral abnormalities were observed. These included erratic swimming, intermittent surfacing, gasping for air, loss of equilibrium, and irregular jerky movements.

At higher concentrations (0.05–0.06 ml/L), the severity of behavioral disturbances increased markedly. Fish exhibited pronounced loss of balance, lateral resting at the bottom, spiraling movements, and rapid opercular activity with an open mouth, indicating respiratory distress and neurotoxic effects. Such behavioral alterations are considered early indicators of toxic stress and impaired physiological functioning in fish exposed to surfactants and quaternary ammonium compounds (Zhang *et al.,* 2022; Yadav *et al.,* 2022) [10, 11].

Table 2: behavioral responses of *Catla catla* fingerlings during exposure to 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	Yes	Yes	No	No	No	No
Erratic swimming and gasping	No	Yes	Yes	Yes	Yes	Yes
Loss of balance	No	No	Yes	Yes	Yes	Yes
Spiraling movements with jerks	No	No	No	Yes	Yes	Yes
Lying laterally at the bottom	No	No	No	No	Yes	Yes
Rapid opercular movement with open mouth	No	No	No	No	No	Yes

Biochemical studies

Glucose

Exposure to Benzalkonium Chloride resulted in a significant elevation in glucose levels in both muscle and gill tissues of *Catla catla*. The glucose content increased from 0.142 mg/g (muscle) and 0.091 mg/g (gills) in control fish to 0.179 mg/g (muscle) and 0.115 mg/g (gills) in exposed fish. This increase indicates stress-induced hyperglycemia, which may be attributed to enhanced glycogenolysis and gluconeogenesis to meet elevated energy demands under toxic conditions (Kumar and Singh, 2021) [5].

Table 3: glucose content in tissues of *Catla catla* to benzalkonium chloride

Tissue	Control (mg/g)	Exposed (mg/g)
Muscle	0.142	0.179
Gills	0.091	0.115

Glycogen

A marked reduction in glycogen content was observed in the tissues of exposed fish. In *Catla catla*, glycogen levels decreased from 0.130 mg/g (muscle) and 0.125 mg/g (gills) in control fish to 0.020 mg/g (muscle) and 0.065 mg/g (gills) following exposure. The depletion of glycogen reserves suggests increased utilization of stored energy to cope with stress induced by Benzalkonium Chloride toxicity (Verma *et al.,* 2023) [9].

Table 4: glycogen content in tissues of *Catla catla* to benzalkonium chloride

Tissue	Control (mg/g)	Exposed (mg/g)
Muscle	0.130	0.020
Gills	0.125	0.065

Protein

Protein content in *Catla catla* showed a significant decline following exposure to Benzalkonium Chloride. Muscle protein levels decreased from 0.660 mg/g in control fish to 0.490 mg/g**,** while gill protein levels decreased from 0.880 mg/g to 0.760 mg/g. This reduction may be attributed to increased proteolysis and the utilization of amino acids for energy production and stress adaptation mechanisms (Sharma and Kaur, 2020) [8].

Table 5: protein content in tissues of *Catla catla* to benzalkonium chloride

Tissue	Control (mg/g)	Exposed (mg/g)
Muscle	0.660	0.490
Gills	0.880	0.760

Histological changes

Gills

Histopathological examination of gill tissues of *Catla catla* exposed to Benzalkonium Chloride revealed significant structural alterations. These included desquamation of the epithelial lining, necrosis (telangiectasia) of the secondary lamellae, shrinkage and distortion of lamellar structures, as well as hypertrophy and hyperplasia at the base of secondary lamellae. Such changes impair respiratory efficiency and indicate severe damage to the gas exchange surfaces.

Muscles

Muscle tissues exhibited notable pathological changes, including nuclear proliferation, variation in muscle fiber size, fragmentation and disintegration of muscle bundles, atrophy, and pronounced thickening and separation of fibers. These alterations reflect impaired muscle integrity and metabolic disturbances caused by toxicant exposure.

Overall, the histological changes observed in both gill and muscle tissues confirm the detrimental effects of Benzalkonium Chloride on cellular architecture and physiological function in *Catla catla* (Zhang *et al.,* 2022; Sharma and Kaur, 2020) [8, 11].

Discussion

The present investigation highlights the acute toxicity and sub-lethal effects of Benzalkonium Chloride, a widely used quaternary ammonium compound, on the freshwater fish *Catla catla*. The results demonstrate that exposure to this chemical induces significant physiological stress, leading to marked biochemical alterations and severe histopathological damage in vital tissues such as gills and muscles.

Biochemical impacts

Glucose level

A significant elevation in glucose levels was observed in both gill and muscle tissues of *Catla catla* exposed to Benzalkonium Chloride. This increase indicates the onset of stress-induced hyperglycemia, which is a common physiological response in fish under toxic conditions. The rise in glucose levels may be attributed to enhanced glycogenolysis and gluconeogenesis, processes that supply immediate energy to cope with toxic stress. Similar findings have been reported in fish exposed to various surfactants and chemical pollutants, confirming that hyperglycemia is a reliable indicator of environmental stress (Kumar and Singh, 2021; Yadav *et al.,* 2022) [5, 10].

Glycogen level

In contrast to glucose, glycogen levels showed a significant decline in the tissues of exposed fish. This depletion suggests that stored glycogen reserves were mobilized to meet increased energy demands under stress conditions. The reduction in glycogen content reflects metabolic imbalance and energy exhaustion caused by Benzalkonium Chloride exposure. Such findings are consistent with earlier studies indicating that toxicants accelerate glycogen breakdown in fish tissues (Verma *et al.,* 2023) [9].

Protein level

A noticeable reduction in protein levels was also recorded in both gill and muscle tissues. Proteins play a crucial role in maintaining cellular structure and metabolic functions; hence, their depletion indicates severe physiological disturbance. The decrease in protein content may result from increased proteolysis and the utilization of amino acids as an alternative energy source during stress. This observation aligns with previous reports where exposure to pollutants led to protein degradation and metabolic disruption in fish (Sharma and Kaur, 2020) [8].

Histopathological effects

Gills

Histological examination of gill tissues revealed extensive structural damage, including epithelial desquamation, necrosis (telangiectasia), shrinkage of secondary lamellae, and hypertrophy and hyperplasia at the base of lamellae. These alterations significantly impair respiratory efficiency and ion regulation. The damage to gill architecture indicates severe disruption of gas exchange mechanisms, leading to hypoxic stress. Similar pathological changes have been documented in fish exposed to quaternary ammonium compounds and other surfactants (Zhang *et al.,* 2022) [11].

Muscles

Muscle tissues exhibited considerable histopathological changes such as atrophy, fragmentation of muscle fibers, variation in fiber size, and thickening and separation of muscle bundles. These alterations suggest degeneration of muscle integrity and impaired physiological functioning. Such damage can adversely affect locomotion, feeding, and predator avoidance, ultimately reducing survival fitness. Comparable findings have been reported in fish exposed to environmental toxicants, indicating muscle tissue sensitivity to chemical stress (Sharma and Kaur, 2020) [8].

Behavioral changes

The behavioral abnormalities observed in *Catla catla*, including erratic swimming, loss of equilibrium, gasping for air, and increased opercular movements, clearly indicate stress and toxicity induced by Benzalkonium Chloride. These responses are often associated with impaired respiratory function and neurological disturbances. Behavioral changes serve as early warning indicators of toxic exposure and correlate well with biochemical and histopathological findings (Yadav *et al.,* 2022; Zhang *et al.,* 2022) [10, 11].

Conclusion

The present study provides a comprehensive evaluation of the toxic effects of Benzalkonium Chloride on the freshwater fish *Catla catla*. Acute toxicity assessment revealed that even low concentrations of this compound can pose significant risks to fish survival, as indicated by the LC_{50} value. Biochemical analyses demonstrated increased glucose levels along with decreased glycogen and protein content, reflecting severe metabolic stress and energy imbalance. Histopathological observations further confirmed extensive damage to gill and muscle tissues, impairing respiratory and locomotory functions.

Overall, the findings highlight the detrimental impact of Benzalkonium Chloride on aquatic organisms and emphasize the need for strict regulation, proper disposal

practices, and increased awareness regarding the environmental risks associated with disinfectants and surfactant-based chemicals. This study contributes to the growing body of knowledge in aquatic toxicology and underscores the importance of protecting freshwater ecosystems from chemical contamination.

Recommendations for future research

1. Long-Term Toxicity Studies

Future investigations should focus on chronic exposure experiments to assess the prolonged effects of Benzalkonium Chloride on the growth, reproduction, and survival of *Catla catla*. Such studies will provide deeper insights into cumulative toxicity and long-term ecological risks.

2. Multi-Species Assessment

Further research should evaluate the impact of Benzalkonium Chloride on a wider range of aquatic organisms, including other freshwater and marine species, to better understand its broader ecological implications and trophic-level effects.

3. Mechanistic and Molecular Studies

Advanced studies are needed to elucidate the cellular and molecular mechanisms underlying Benzalkonium Chloride-induced toxicity, including oxidative stress pathways, enzyme alterations, and gene expression changes. This will help in identifying biomarkers and developing targeted mitigation strategies.

4. Environmental Monitoring and Risk Assessment

Regular monitoring of water bodies for the presence of Benzalkonium Chloride and similar surfactants should be implemented. This will aid in early detection of contamination, environmental risk assessment, and formulation of effective pollution control measures.

5. Development of Eco-Friendly Alternatives

Research should also focus on developing and promoting environmentally safe and biodegradable alternatives to chemical disinfectants to minimize ecological damage.

This study emphasizes the environmental risks associated with the widespread use of Benzalkonium Chloride and highlights the urgent need for sustainable practices, proper waste management, and regulatory measures to safeguard aquatic ecosystems and species such as *Catla catla*.

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