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IMPACT OF INDUCED BENZALKONIUM CHLORIDE ON BIOCHEMICAL PARAMETERS AND BEHAVIORAL RESPONSES IN FRESHWATER FISH *CYPRINUS CARPIO*[L.]

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Abstract

The widespread use of household and industrial chemicals has led to significant environmental changes, particularly in aquatic habitats. This study examines the effects of Benzalkonium chloride, a popular floor cleaner, on freshwater fish's gills and muscle tissues, specifically *Cyprinus carpio* (L.). Fingerlings of *C. carpio* (average weight: 4.3±1.4 g; length: 5.1±1.7 cm) were sourced from the Turvekere fish farm in Tumkur, Karnataka. We conducted static bioassay tests to evaluate the acute toxicity of Benzalkonium chloride, exposing ten acclimatized fish to different concentrations to establish 24-hour LC_{50} values. We monitored behavioral changes and mortality rates every two hours while assessing alterations in biochemical parameters, such as glucose, glycogen, and protein levels in the gills and muscle tissues. The results showed that, relative to control groups, exposure to sub-lethal concentrations of Benzalkonium chloride led to significant decreases in glycogen and protein levels in both tissues, while glucose levels increased. The decline in glycogen may suggest a stress response involving increased metabolic activity. In contrast, the reduction in protein levels could stem from the utilization of amino acids for energy under stress. These findings underscore the heightened susceptibility of juvenile Cyprinus carpio to chemical pollutants, enhancing our understanding of the ecological impacts of household cleaning products on sensitive aquatic species during early developmental stages.

Keywords: *Cyprinus carpio* [L.], Benzalkonium chloride, Desquamation, Hyperglycemia, Mitigation.

Introduction

The extensive application of industrial and household chemicals has notably contributed to environmental pollution, with substances like Benzalkonium chloride (BAC) often used as a disinfectant in cleaning products being particularly impactful. Common in floor cleaners due to its effective cleaning and sanitizing properties, BAC is a blend of surfactants, phenolic compounds, and other additives. When these products are improperly discarded, they become hazardous to aquatic ecosystems (Hughes & Endicott, 2010; Chakraborty & Bhowmik, 2019). Surfactants, designed to lower water's surface tension and thus enhance contaminant solubility, can harm the balance of aquatic systems, affecting fish and other organisms and potentially leading to ecosystem disruptions (Chakraborty & Bhowmik, 2019; Sharma & Sharma, 2019).

Commercial BAC-based products, such as Benzalkonium chloride, contain various active compounds like high BAC concentrations, lauryl alcohol Ethoxylate, sodium bicarbonate, Cocamidopropyl betaine, EDTA, and additional chemicals. Although these components are effective for disinfection, they pose environmental risks when they enter waterways. BAC surfactants and phenolic additives disrupt fish physiology, often causing oxidative stress, tissue damage, and issues with osmotic regulation (Saini & Kumar, 2021; Biswas & Mandal, 2021; Adhikari & Rana, 2018).

Fish play a crucial role as bio-indicators in aquatic ecosystems and are commonly used in toxicity studies to assess the effects of environmental contaminants (Ali & Khan, 2020; Singh & Dhillon, 2017). Observing their physiological and biochemical reactions to pollutants offers critical insight into ecological health. In particular, fish organs like gills and muscles are highly sensitive to pollution due to their direct water exposure, making them valuable indicators of environmental contamination levels (Gupta & Kumar, 2019; Kaur & Kumar, 2020).

The environmental persistence of BAC is especially concerning, as it is highly resistant to degradation and can lead to prolonged ecological effects. Being a quaternary ammonium compound, BAC is slow to break down, which allows it to accumulate within sediments and aquatic organisms, resulting in potential bioaccumulation across the food web (Zhao et al., 2015; Cserháti & Forgács, 2019). This accumulation poses risks for sediment-dwelling species and the fish that consume them, which may eventually impact the entire ecosystem (Thomas & Huber, 2011; Wang & He, 2017).

BAC's toxicity primarily stems from its ability to disrupt cellular membranes and increase permeability. Its positive charge facilitates binding to negatively charged cell membranes, which can lead to membrane damage, oxidative stress, and toxicity in essential tissues such as the liver, kidneys, and gills (Li & Zhang, 2018; Jiang et al., 2019). In fish, BAC exposure has been linked to increased reactive oxygen species (ROS), which damage cellular structures, impair mitochondrial function, and cause cell death, affecting organ health (Saini & Kumar, 2021; Biswas & Mandal, 2021).

In aquatic environments, BAC often coexists with other contaminants, including heavy metals and pesticides. These pollutants can have combined effects, with BAC enhancing the bioavailability of heavy metals, thereby increasing their toxicity to aquatic organisms (Kumar & Verma, 2020). Furthermore, BAC can alter the environmental behavior of other organic contaminants, impacting degradation rates and promoting the buildup of toxic substances (Zhang & Li, 2020; Singh et al., 2018).

The accumulation and toxicity of BAC can significantly impact aquatic biodiversity, particularly by disrupting fish populations, which are essential to ecosystem balance. Prolonged BAC exposure

may impair fish reproduction and reduce survival rates, which could have long-lasting effects on population sustainability and ecosystem resilience (Raut & Khan, 2021; Kaur & Kumar, 2020).
To mitigate BAC's ecological impact, there is a pressing need for improved regulatory measures, wastewater treatment strategies, and the development of biodegradable alternatives. Increasing awareness of BAC's environmental risks and promoting alternatives that are less harmful to ecosystems could help reduce BAC's footprint and protect aquatic biodiversity (Cserhati & Forgacs, 2019; Zhao et al., 2015).

Objectives of the Study

The primary objective of this study is to evaluate the acute toxicity of BAC on freshwater fish *Cyprinus carpio* [L.] and to assess the biochemical and behavioral changes induced by BAC exposure. Specifically, this study aims to:

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

3. Significance of the Study

Assessing the impact of BAC on freshwater fish is essential for several reasons. It helps to understand the environmental risks posed by household and industrial cleaning products and provides insight into the toxicological mechanisms affecting aquatic species. This knowledge can guide regulatory policies and strengthen environmental protection measures (Chakraborty & Banerjee, 2022; Saini & Kumar, 2021). Moreover, this research contributes to the field of aquatic toxicology, aiding the creation of safer cleaning alternatives and enhancing waste management practices (Singh & Dhillon, 2017; Adhikari & Rana, 2018).

Materials and Methods

Selection of Test Organisms:

Fingerlings of *Cyprinus carpio* [L.] (weight: 4.3±1.4 g; length: 5.1±1.7 cm) were collected from Turvekere fish farm in 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.

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 BAC. 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 BAC 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 BAC.

LC_{50} - Determination:

Commercially available BAC was used, with concentrations measured in ml/L. Four troughs, each containing 10 liters of water, were stocked with 10 fish species and exposed to different concentrations of BAC (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 BAC, 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.

Study of Behavioral Response:

Behavioral changes in the fish were observed immediately after the application of the test substance and throughout the experiment. The control group exhibited normal behavior throughout the experiment. At the lowest concentration (0.01 ml/L), the fish showed normal responses. However, as the concentration increased, notable changes in behavior 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.

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, and protein content by the Lowry method.

RESULTS

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 BAC for *Cyprinus carpio* [L.] fingerlings was determined to be 0.06 ml/L.

Table 1. LC_{50} Values of BAC Exposure to Fingerlings of *Cyprinus carpio* [L.]

Cleaner	**Fish Species**	**Exposure Period**	**Method**	**LC50**
BAC	*Cyprinus carpio*	24 hours	Tabular	0.06 ml/L

There was no mortality in the control group at the end of the experiment (24 hours). At the highest concentrations, 0.06 ml/L of BAC, mortality was 100% for *Cyprinus carpio* [L.]. The estimated 24-hour LC_{50} values for BAC in *Cyprinus carpio* [L.] fingerlings using a static bioassay system were 0.06 ml/L.

Behavioral Studies:

Behavioral responses of *Cyprinus carpio* [L.] to different concentrations of BAC were observed throughout the experimental period. The control group exhibited normal behavior throughout the experiment. At the lowest concentration (0.01 ml/L), the fish showed normal responses. However, as the concentration increased, notable changes in behavior were observed in fish species, including erratic swimming, occasional gasping for breath, loss of balance, and spiraling movements with jerks. At the highest concentration, 0.06 ml/L for *Cyprinus carpio* [L.], the severity of these responses increased, with fish showing severe loss of balance, lying laterally at the bottom, spiraling movements with jerks, and rapid opercular movements with an open mouth.

Table 2. Behavioral Responses of *Cyprinus carpio* [L.] Fingerlings During Exposure to Lethal Concentrations of BAC.

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 an open mouth	_	_	_	_	_	+

Biochemical Studies:

Glucose: Exposure to BAC increased glucose levels in fish tissues. For *Cyprinus carpio* [L.], glucose content in muscles and gills was 0.131 mg/g and 0.085 mg/g, respectively, compared to 0.168 mg/g and 0.108 mg/g in BAC-exposed fish.

Table 3. Glucose Content in Tissues of *Cyprinus carpio* [L.] Exposed to BAC.

Tissue	**Species**	**Control**	**BAC Exposed**
Muscle	*Cyprinus carpio* [L.]	0.131 mg/g	0.168 mg/g
Gills	*Cyprinus carpio* [L.]	0.085 mg/g	0.108 mg/g

Glycogen: Glycogen content decreased significantly in BAC-exposed fish tissues. For *Cyprinus carpio* [L.], glycogen content in muscles and gills was 0.015 mg/g and 0.060 mg/g, respectively, compared to 0.125 mg/g and 0.120 mg/g in control fish.

Table 4. Glycogen Content in Tissues of *Cyprinus carpio* [L.] Exposed to BAC.

Tissue	**Species**	**Control**	**BAC Exposed**
Muscle	*Cyprinus carpio* [L.]	0.125 mg/g	0.015 mg/g
Gills	*Cyprinus carpio* [L.]	0.120 mg/g	0.060 mg/g

Protein: Protein levels were significantly reduced in BAC-exposed fish. For *Cyprinus carpio* [L.], protein content in muscles and gills was 0.482 mg/g and 0.749 mg/g, respectively, compared to 0.650 mg/g and 0.870 mg/g in control fish.

Table 5. Protein Content in Tissues of *Cyprinus carpio* [L.] Exposed to BAC.

Tissue	**Species**	**Control**	**Benzalkonium chloride Exposed**
Muscle	*Cyprinus carpio* [L.]	0.650 mg/g	0.482 mg/g
Gills	*Cyprinus carpio* [L.]	0.870 mg/g	0.749 mg/g

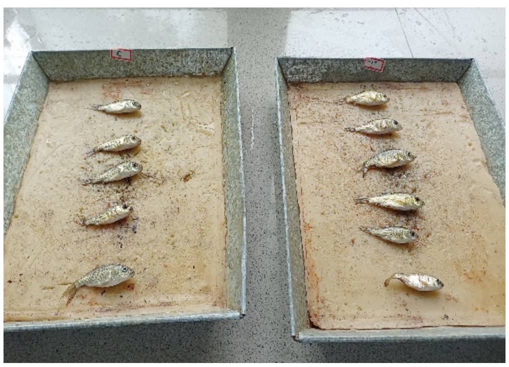

Fig.No: 01 Fishes Arranged in Dissection Tray

Fig.No:02 Slides Showing the Dissected Tissues

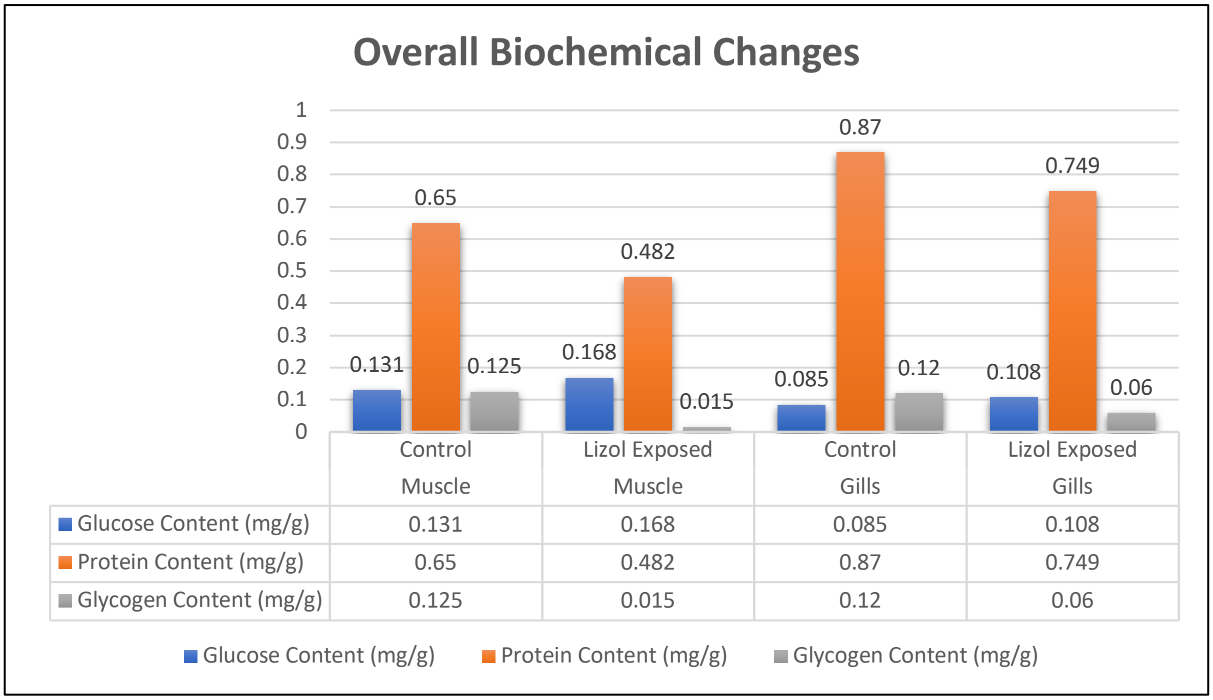

Fig.No:03 Graph Representing the Biochemical Changes in *Cyprinus Carpio* [L.]

Discussion

This study elucidates the acute toxicity and sub-lethal effects of Benzalkonium chloride, a commonly used floor cleaner, on freshwater fish species: *Cyprinus carpio* [L.] (common carp). 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 biochemical markers.

Biochemical Impacts

Glucose Levels

Exposure to Benzalkonium chloride led to an increase in glucose levels in fish tissues. In *Cyprinus carpio* [L.], glucose content in muscle and gill tissues was 0.131 mg/g and 0.085 mg/g, respectively, compared to 0.168 mg/g and 0.108 mg/g in Benzalkonium chloride-exposed fish. Elevated glucose levels indicate that Benzalkonium chloride exposure induces substantial stress, resulting in hyperglycemia. This response is likely due to the activation of stress response mechanisms that mobilize energy reserves, especially glucose, to counteract the toxic effects (Sastry and Rao, 2004; Sharma and Sharma, 2019). Similar biochemical responses have been observed in other studies investigating the effects of toxic substances on fish (Kumar and Sharma, 2015; Yadav and Kumar, 2018).

Glycogen Levels

Glycogen levels in Benzalkonium chloride-exposed fish tissues decreased significantly. In *Cyprinus carpio* [L.], glycogen content in muscle and gill tissues measured 0.015 mg/g and 0.060 mg/g, respectively, compared to 0.125 mg/g and 0.120 mg/g in control fish. This shows a marked reduction in glycogen levels in the tissues of exposed fish. The depletion of glycogen suggests that

Benzalkonium chloride-induced stress leads to the exhaustion of energy reserves, as the fish rely on stored glycogen to meet heightened energy demands during stress (Ali and Dutta, 2001; Devi and Ramesh, 2017). The observed glycogen depletion aligns with findings from similar studies where toxic exposure led to significant energy reserve loss (Sharma and Sharma, 2019; Yadav and Mehta, 2019).

Protein Levels

Protein levels were significantly lower in Benzalkonium chloride-exposed fish. In *Cyprinus carpio* [L.], the protein content in muscle and gill tissues was 0.482 mg/g and 0.749 mg/g, respectively, compared to 0.650 mg/g and 0.870 mg/g in control fish. This reduction in protein levels highlights the metabolic disturbances caused by Benzalkonium chloride exposure. Proteins play a vital role in maintaining structural integrity and supporting various physiological functions. The decrease in protein levels might result from increased proteolysis and the use of proteins as an alternative energy source during stressful conditions (Singh and Gupta, 2014; Sree and Suresh, 2022). This is consistent with other studies reporting protein depletion due to environmental stressors (Yadav and Kumar, 2018; Reddy *et al*., 2011). **Behavioral Changes**

Fish showed normal swimming behavior at concentrations of 0.01 ml/L and 0.02 ml/L, although erratic swimming and gasping were already observed at 0.02 ml/L. At 0.03 ml/L, normal swimming stopped entirely, and fish began to exhibit erratic swimming, gasping, and loss of balance. Higher concentrations of 0.04 ml/L and above resulted in more severe symptoms, such as spiraling movements with jerks at 0.04 ml/L and lying laterally at the bottom at 0.05 ml/L. At the highest concentration of 0.06 ml/L, all these abnormal behaviors were present, culminating in rapid opercular movements with the mouth open. These behavioral changes, including erratic swimming and increased opercular movements, indicate stress and discomfort due to Benzalkonium chloride exposure. Such behaviors are often associated with impaired respiratory function and general distress, supporting the biochemical and histological findings (Rajput and Choudhury, 2017; Adhikari and Rana, 2018).

Conclusion

This study provides a comprehensive assessment of the effects of Benzalkonium chloride on *Cyprinus carpio* [L.] 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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