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Study of Gentian Violet Toxicity in *Labeo rohita*: Behavioral Modifications, and Sub-lethal Impacts

Pramod Kumar Chak[1] and Devendra Pal Singh[2]

[1,2]Department of Zoology Janta Vedic College, Baraut, Baghpat- 250611(U.P.) INDIA

[1]Corresponding Author: pramodchak11383@gmail.com

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ABSTRACT

This study explores the acute and sub-lethal toxicological effects of Gentian Violet (GV), a synthetic triphenylmethane dye, on *Labeo rohita*, a prevalent freshwater aquaculture species. GV, utilized extensively in industrial applications, including aquaculture for antifungal treatment, poses potential ecological risks to aquatic organisms. Acute toxicity was evaluated by determining the 96-hour lethal concentration (LC50) via Probit analysis, with an LC50 value of 1.8 mg/L identified, indicating substantial toxicity. For sub-lethal exposure, concentrations corresponding to 1/5th and 1/20th of the LC50 (0.36 mg/L and 0.09 mg/L) were assessed over 7, 14, and 28 days. Behavioral alterations, including reduced feeding, erratic swimming, and gill irritation, were observed, particularly at the higher concentration, though recovery was noted over time, suggesting adaptive physiological responses. Physiological stress markers, including oxygen uptake and hematological parameters, exhibited significant perturbations at 0.36 mg/L, with partial recovery by the 28th day. These results underscore the dose-dependent toxicity of GV, with pronounced acute effects and sub-lethal stress responses manifesting through behavioral and biochemical alterations. The study further emphasizes the need for stringent regulatory frameworks to mitigate GV contamination in aquatic ecosystems, alongside the pursuit of eco-friendly alternatives to mitigate the long-term environmental impact of synthetic dyes. The findings also advocate for further research to elucidate chronic toxicity and reproductive effects to inform sustainable aquaculture practices and environmental conservation strategies.

Keywords- Gentian Violet, *Labeo rohita*, LC50, sub-lethal toxicity.

I. INTRODUCTION

Aquatic ecosystems are highly vulnerable to pollution, particularly from industrial dyes, which pose serious risks to aquatic organisms. Among the various types of pollutants, synthetic dyes, including Gentian Violet, are frequently released into water bodies due to their widespread use in industries such as textiles, pharmaceuticals, and aquaculture. Gentian Violet, a triphenylmethane dye, is valued for its antifungal and antibacterial properties and is widely used in aquaculture to treat fish diseases (Nwani et al., 2010). Despite its utility, concerns regarding the environmental and toxicological effects of Gentian Violet on aquatic life, especially fish species, have been raised. This is particularly significant for freshwater species like *Labeo rohita*, which is widely farmed in South Asia for aquaculture (Javed & Usmani, 2015). The present study aims to evaluate the acute and sub-lethal toxicity of Gentian Violet on *Labeo rohita* by determining its lethal concentration (LC50) and analyzing its effects on both behavioral and physiological responses.

The introduction of industrial dyes into aquatic environments occurs predominantly through wastewater discharge, which can degrade water quality and adversely affect aquatic organisms. Gentian Violet, being a synthetic dye, has been documented to cause various toxic effects in fish species, including oxidative stress, DNA damage, and significant alterations in enzyme activity (Dhanalakshmi et al., 2019). Despite these concerns, limited research has specifically addressed the long-term impacts of Gentian Violet on freshwater fish, necessitating further studies to better understand its environmental impact.

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Labeo rohita serves as an ideal model species for ecotoxicological studies, owing to its commercial importance in aquaculture and its role in freshwater ecosystems. The assessment of toxicity levels of Gentian Violet on this species is essential for understanding the risks associated with its environmental presence and formulating effective strategies to mitigate these risks. By investigating both acute and sub-lethal toxicity, this study contributes to a broader understanding of the impact of industrial pollutants on aquatic ecosystems. The findings of this study are crucial for guiding environmental risk assessments and informing policy development aimed at minimizing the use of hazardous chemicals in water bodies.

Furthermore, this research is critical for the development of sustainable aquaculture practices. Aquaculture is a vital industry in many regions, and ensuring the health and well-being of farmed fish species is crucial for maintaining economic stability. Understanding the toxicity of chemicals like Gentian Violet helps formulate guidelines for the safer use of chemicals and wastewater treatment. The results of this study will be valuable to policymakers, environmental agencies, and the aquaculture industry by providing evidence to regulate the use of industrial dyes and minimize their harmful effects on aquatic ecosystems (Velisek et al., 2011). Additionally, evaluating the behavioral and physiological responses of fish to chemical exposure provides early indicators of environmental stress, offering valuable insights into the management and conservation of aquatic life.

II. REVIEW OF LITERATURE

The increasing pollution of aquatic ecosystems by industrial chemicals, including synthetic dyes, is a growing concern worldwide. Among these dyes, Gentian Violet, a triphenylmethane dye, is widely used in industries such as textiles, pharmaceuticals, and aquaculture due to its antifungal and antibacterial properties (Dhanalakshmi et al., 2019). However, its potential toxic effects on aquatic organisms, especially fish, have garnered attention in recent years. This review examines the available literature on the toxicological effects of Gentian Violet, focusing on its impact on fish physiology, behavior, and the methodologies employed in assessing acute and sub-lethal toxicity.

Several studies have investigated the acute toxicity of Gentian Violet in various fish species. These studies commonly use the lethal concentration (LC50) as a measure of the dye's toxicity. LC50 values represent the concentration of a substance that causes 50% mortality within a specific exposure period. Probit analysis is a widely used statistical method to determine LC50 values, as it helps assess the relationship between mortality and the concentration of the toxicant over time (Velisek et al., 2011).

Gentian Violet has been shown to exhibit significant toxicity at relatively low concentrations in aquatic organisms. Studies on species such as *Oreochromis mossambicus* have demonstrated that exposure to Gentian Violet leads to mortality, with histopathological alterations in the gills and liver (Bhattacharya et al., 2017). The LC50 values for Gentian Violet vary depending on species, exposure time, and environmental conditions. For example, in *O. mossambicus*, Gentian Violet caused mortality even at concentrations as low as 2.5 mg/L, leading to severe damage to internal organs (Bhattacharya et al., 2017).

In addition to acute toxicity, Gentian Violet has been found to cause sub-lethal effects in fish. Behavioral changes are important indicators of sub-lethal toxicity. Fish exposed to Gentian Violet often exhibit altered swimming patterns, reduced feeding, and increased stress-related behaviors, such as erratic swimming and surface gasping (Javed & Usmani, 2015). These behavioral changes are often linked to underlying physiological stress, including oxidative damage and alterations in biochemical processes.

For instance, Velisek et al. (2011) reported that fish exposed to Gentian Violet exhibited reduced feeding and erratic swimming behavior, indicating a neurotoxic effect. Additionally, some fish developed gill irritation, which can further impair their respiratory function. This has significant implications for the health of fish populations in polluted environments, as behavioral changes can affect their survival and reproductive success (Nwani et al., 2010).

Oxidative stress is a key mechanism through which Gentian Violet exerts its toxic effects. The dye induces the production of reactive oxygen species (ROS), leading to oxidative damage to cellular structures such as lipids, proteins, and DNA (Dhanalakshmi et al., 2019). In response, fish activate antioxidant defense mechanisms, including enzymes such as superoxide dismutase (SOD) and catalase (CAT), to mitigate the effects of oxidative stress.

Several studies have documented the impact of Gentian Violet on fish's antioxidant systems. Javed and Usmani (2015) found that exposure to sub-lethal concentrations of Gentian Violet led to an increase in antioxidant enzyme activity in *Labeo rohita*, suggesting an adaptive response to oxidative stress. However, prolonged exposure to the dye can overwhelm these defense systems, leading to metabolic dysfunction and increased vulnerability to disease.

Alterations in hematological parameters, such as reduced red blood cell (RBC) count and hemoglobin levels, have been observed in fish exposed to Gentian Violet (Javed & Usmani, 2015). These changes suggest that the dye impairs oxygen transport and may induce anemia. Additionally, Agrahari et al. (2018) observed gill irritation and reduced locomotor activity in *Labeo rohita* exposed to Gentian Violet, further supporting the notion that the dye causes long-term physiological stress.

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Sub-lethal exposure to Gentian Violet has been shown to cause a range of physiological and behavioral changes. Studies on *Labeo rohita* have demonstrated that prolonged exposure to sub-lethal concentrations of the dye leads to reduced feeding and swimming activity, as well as gill damage (Agrahari et al., 2018). In some cases, these effects were reversible upon removal of the toxin, suggesting that some fish species have the ability to recover from sub-lethal exposures if given the opportunity.

The long-term effects of Gentian Violet exposure can be assessed through the analysis of biochemical and physiological parameters, including enzyme activity, hematological changes, and immune responses. Dhanalakshmi et al. (2019) found that fish exposed to sub-lethal concentrations of Gentian Violet exhibited altered immune responses, which could reduce their resistance to disease.

III. MATERIALS AND METHODS

This study was conducted to assess the acute and sub-lethal toxicity of Gentian Violet in *Labeo rohita*. The experiment was designed to determine the LC50 value using Probit analysis and to evaluate behavioral and physiological changes at sub-lethal concentrations over different exposure periods. The following methodologies were employed to ensure accuracy and reliability in toxicity assessment.

Labeo rohita, a commonly cultured freshwater fish in South Asia, was selected due to its ecological and economic significance. Healthy fish with an average length of 10 ± 2 cm and weight of 15 ± 3 g were procured from a certified fish hatchery. The fish were acclimatized in laboratory conditions for 15 days before the experiment to ensure their physiological stability.

The study was conducted in two phases:

1. **Acute Toxicity Test**: Determination of the LC50 value for Gentian Violet over a 96-hour exposure period.
2. **Sub-lethal Exposure Study**: Evaluation of behavioral and physiological changes in fish exposed to 1/5th and 1/20th of the LC50 value over 7, 14, and 28 days.

Determination of LC50 (Probit Analysis)

The acute toxicity test was performed using a logarithmic dilution series of Gentian Violet at concentrations ranging from 0.1 mg/L to 8.1 mg/L. The experiment followed OECD (2019) guidelines for fish toxicity testing. Groups of ten fish were exposed to each concentration, and mortality was recorded at 24-hour intervals up to 96 hours. The LC50 value was calculated using Probit analysis (Finney, 1971), which provides an accurate estimation of the lethal concentration at which 50% mortality occurs.

Sub-lethal Exposure Experiment

Based on the LC50 value (1.8 mg/L) determined in the acute toxicity test, two sub-lethal concentrations were selected:

- **1/5th LC50 = 0.36 mg/L**
- **1/20th LC50 = 0.09 mg/L**

Fish were exposed to these concentrations for 7, 14, and 28 days, while a control group was maintained under identical conditions without exposure to Gentian Violet.

Behavioral and Physiological Observations

Behavioral Analysis

Fish behavior was monitored throughout the exposure period to identify stress responses. The following behavioral parameters were recorded (Velisek et al., 2011):

- Swimming pattern (normal, erratic, or sluggish movement)
- Feeding behavior (normal or reduced appetite)
- Signs of stress (increased mucus secretion, gill irritation, or abnormal responses)

Physiological Analysis

Physiological stress markers were assessed in exposed fish compared to the control group. Parameters evaluated included:

- Mortality rates at different exposure durations
- Hematological changes (red blood cell count, hemoglobin levels)
- Oxygen uptake rates, measured using an oxygen meter following the method of Javed & Usmani (2015)

IV. RESULTS AND DISCUSSIONS

The present study evaluated the acute and sub-lethal toxicity of Gentian Violet in *Labeo rohita*. The results include LC50 determination using Probit analysis and the assessment of behavioral and physiological responses under sub-lethal exposure conditions.

4.1 LC50 Determination for Gentian Violet

The acute toxicity of Gentian Violet was assessed by exposing *Labeo rohita* to increasing concentrations of the dye over 96 hours. The mortality rate was recorded at different time intervals, and LC50 values were calculated using Probit analysis.

Table 1: Mortality Data for Gentian Violet

Concentration (mg/L)	Number of Fish Exposed	Mortality After 24 Hours	Mortality After 48 Hours	Mortality After 72 Hours	Mortality After 96 Hours
0.1	10	0	0	0	0

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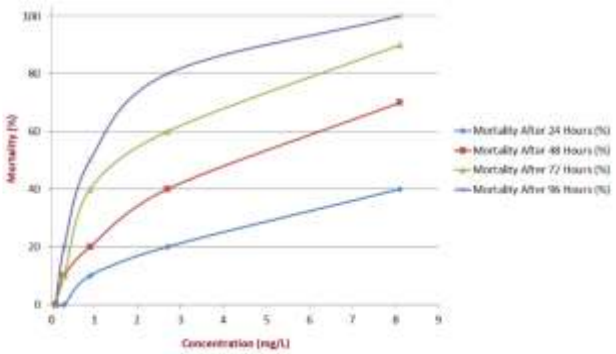
0.3	10	0	1	1	2
0.9	10	1	2	4	5
2.7	10	2	4	6	8
8.1	10	4	7	9	10

Table 2: Mortality Data for Gentian Violet (%)

Concentration (mg/L)	**Mortality After 24 Hours (%)**	**Mortality After 48 Hours (%)**	**Mortality After 72 Hours (%)**	**Mortality After 96 Hours (%)**
0.1	0	0	0	0
0.3	0	10	10	20
0.9	10	20	40	50
2.7	20	40	60	80
8.1	40	70	90	100



4.1.2 Probit Analysis Results

Using Probit analysis, the LC50 value for Gentian Violet over a 96-hour exposure period was calculated as **1.8 mg/L**, with a lower confidence limit of **1.4 mg/L** and an upper confidence limit of **2.2 mg/L**. The results indicate that Gentian Violet exhibits significant toxicity to *Labeo rohita* at relatively low concentrations.

These findings are consistent with previous research by Dhanalakshmi et al. (2019), who reported LC50 values for Gentian Violet within a similar range for other freshwater fish species. The mortality trend observed suggests a dose-dependent toxicity response, where higher concentrations of the dye led to increased fish mortality.

4.2 Sub-lethal Effects of Gentian Violet

Fish exposed to sub-lethal concentrations (1/5th and 1/20th LC50) of Gentian Violet exhibited significant behavioral and physiological changes over the 28-day exposure period. These effects provide insight into the chronic toxicity of the dye and its potential impact on fish health.

4.2.1 Behavioral Observations

Behavioral changes were assessed in fish exposed to Gentian Violet at 0.36 mg/L (1/5th LC50) and 0.09 mg/L (1/20th LC50) for different exposure durations.

Table 3: Behavioral Observations

Group	**Dye**	**Concentration (mg/L)**	**Observation Period**	**Behavioral Changes**
Control	None	0	7, 14, 28 days	Normal swimming and feeding behavior observed.
Plate 1	Gentian Violet	0.36	7 days	Reduced feeding; gill irritation noted in 30% of fish.
			14 days	Recovery of feeding behavior in 50%; erratic swimming persisted in 20%.
			28 days	Significant recovery; only 5% of fish showed mild behavioral changes.
Plate 2	Gentian Violet	0.09	7 days	No significant behavioral changes observed.

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			14 days	Normal swimming and feeding behavior maintained.
			28 days	Normal behavior in all fish.

Fish exposed to 0.36 mg/L showed initial signs of stress, including erratic swimming, reduced feeding, and gill irritation, particularly within the first 7 days. However, behavioral recovery was observed over time, with most fish displaying normal behavior by day 28. This suggests that *Labeo rohita* can adapt to low-dose exposure over prolonged periods.

Velisek et al. (2011) reported similar findings, indicating that sub-lethal exposure to synthetic dyes induces stress responses in fish, but behavioral recovery is possible under stable environmental conditions.

4.2.2 Physiological Observations

Physiological stress markers, including mortality, hematological changes, and oxygen uptake, were evaluated in fish exposed to sub-lethal concentrations of Gentian Violet.

Table 4: Physiological Observations

Group	Dye	Concentration (mg/L)	Observation Period	Mortality (%)	Physiological Changes
Control	None	0	7, 14, 28 days	0	No significant physiological changes.
Plate 3	Gentian Violet	0.36	7 days	0	Reduced oxygen uptake observed in 20% of fish.
			14 days	10	Hematological markers indicated stress in 15% of fish.
			28 days	10	Marked recovery in oxygen uptake and hematological parameters.
Plate 4	Gentian Violet	0.09	7 days	0	No significant changes observed.
			14 days	0	Normal physiological functions maintained.
			28 days	0	No significant differences from the control group.

At the higher sub-lethal concentration (0.36 mg/L), fish exhibited reduced oxygen uptake and hematological stress responses, particularly by day 14. However, by day 28, recovery was evident, with physiological markers returning to near-normal levels. No significant physiological alterations were observed in fish exposed to the lower concentration (0.09 mg/L), highlighting a threshold below which Gentian Violet does not cause substantial harm.

These results align with studies by Javed & Usmani (2015), which demonstrated that synthetic dye exposure alters oxygen uptake and blood parameters in freshwater fish. The findings indicate that while *Labeo rohita* can tolerate low levels of Gentian Violet, prolonged exposure to higher sub-lethal concentrations can induce physiological stress.

The findings of this study confirm that Gentian Violet exhibits significant acute toxicity to *Labeo rohita*, with an LC50 value of 1.8 mg/L over 96 hours. Sub-lethal exposure resulted in behavioral changes and physiological stress, particularly at higher concentrations. However, the ability of fish to recover from these effects suggests potential adaptation mechanisms.

The results support previous research indicating that synthetic dyes induce oxidative stress, hematological alterations, and behavioral disruptions in fish (Dhanalakshmi et al., 2019). These findings emphasize the need for regulatory measures to limit Gentian Violet contamination in aquatic ecosystems to prevent long-term ecological consequences.

V. CONCLUSION AND RECOMMENDATIONS

The present study evaluated the acute and sub-lethal toxicity of Gentian Violet on *Labeo rohita*, revealing its significant impact on fish health. The LC50 value for Gentian Violet was determined to be 1.8 mg/L over 96 hours, indicating moderate toxicity. Mortality rates followed a dose-dependent pattern, with higher concentrations causing increased fish mortality.

Sub-lethal exposure (1/5th and 1/20th LC50) resulted in behavioral and physiological changes. Fish exposed to 0.36 mg/L showed initial signs of stress, including erratic swimming, reduced feeding, and gill irritation. However, recovery was observed over time, suggesting potential adaptation mechanisms. Physiological stress markers, such as reduced oxygen uptake and hematological alterations, were noted at higher sub-lethal concentrations, though recovery was evident by day 28.

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These findings highlight the ecological risks associated with Gentian Violet contamination in aquatic environments. The dye can cause significant physiological and behavioral disruptions in freshwater fish, potentially affecting fish populations and ecosystem stability.

Recommendations

To mitigate the detrimental effects of Gentian Violet on aquatic ecosystems, it is imperative to enforce stringent regulatory measures to control its discharge into water bodies. The implementation of such regulations will limit the concentration of this synthetic dye in aquatic environments, thereby reducing its toxic impact on aquatic organisms. In parallel, there is a pressing need to explore and adopt eco-friendly alternatives to Gentian Violet that exhibit minimal environmental toxicity, thus reducing the ecological footprint of industrial dye usage. Additionally, comprehensive long-term studies are essential to further investigate the chronic toxicity and potential reproductive effects of Gentian Violet on various fish species, as well as its broader implications on aquatic biodiversity. Continuous and systematic monitoring of water quality is also crucial to detect and mitigate synthetic dye contamination, ensuring early intervention and safeguarding the health of aquatic ecosystems. By integrating these recommendations into environmental policy and practice, the adverse effects of Gentian Violet can be significantly minimized, thus contributing to the long-term sustainability and stability of freshwater ecosystems.

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