Sub-Lethal Toxicity of Paraquat Dichloride (200g/L) on Biochemical Properties of Clarias Gariepinus Juveniles

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ABSTRACT

This study investigates the sub-lethal effects of varying concentrations of Paraquat dichloride on biochemical parameters and mortality rates of juvenile Clarias gariepinus over a 28-day exposure period. A controlled laboratory experiment was conducted with five treatment groups (T1 to T5) of juvenile C. gariepinus, each comprising nine fish. The control group (T1) received no Paraquat, while T2 to T5 were exposed to increasing concentrations (0.034 to 0.134 ml/L) of Paraquat dichloride (200 g/L). Mortality rates increased significantly with higher Paraquat concentrations, peaking at 66.67% in T5. Results also revealed the biochemical disruptions caused by Paraquat exposure indicated increased glucose levels, liver dysfunction and altered cholesterol metabolism in C. gariepinus, suggesting physiological stress and toxicity of juvenile C. gariepinus leading to elevated mortality and compromised health. This study emphasized the critical role of biochemical assessments in evaluating health impacts of pollutants on fish.

Keywords: Sub-lethal toxicity, C. gariepinus, Paraquat, Biochemical Assessments.

INTRODUCTION

Paraquat dichloride is widely used as non-selective herbicide known for its effectiveness in controlling a broad spectrum of weeds in agriculture (González et al., 2020). However, its extensive use presents significant environmental risks, particularly to non-target organisms in aquatic ecosystems. Runoff from agricultural fields often introduces Paraquat into nearby water bodies, posing a threat to aquatic life. Among the aquatic organisms at risk is Clarias gariepinus (African catfish), an ecologically and economically important freshwater species, especially in Nigeria. Its adaptability to diverse environmental conditions makes it a cornerstone of local aquaculture (FAO, 2022). Despite its resilience, C. gariepinus is particularly vulnerable to environmental pollutants, which can compromise its health, disrupt physiological functions, and threaten population stability. While acute toxicity studies have traditionally assessed pesticide hazards, increasing attention is being directed toward sub-lethal toxicity. Sub-lethal exposure may not cause immediate mortality but can induce physiological impairments that affect growth, reproduction, and behaviour over time (Kumar et al., 2019). These sub-lethal effects often manifest at biochemical level, where alterations in enzyme activity, metabolic markers, and stress responses can signal early signs of toxicant-induced damage.

Previous research indicates that pesticides like Paraquat can disrupt metabolic processes and elevate oxidative stress in fish (Mansour et al., 2021). For C. gariepinus, such disruptions may reduce reproductive success and compromise immune function, potentially diminishing both individual fitness and population viability. Despite this, there remains a gap in comprehensive studies addressing the biochemical impacts sub-lethal Paraquat exposure on juvenile C. gariepinus. This knowledge gap limits the ability to conduct comprehensive ecological risks assessments and to design effective mitigation strategies.

This study aims to bridge that gap by evaluating the sub-lethal effects of Paraquat dichloride (200 g/L) on the biochemical parameters and mortality rates of juvenile Clarias gariepinus juveniles over 28 days. Paraquat poses substantial risks to non-target aquatic organisms, and understanding its effects is crucial for developing effective mitigation strategies (González et al., 2020).

Investigating the biochemical responses of Clarias gariepinus juveniles offer insights into the health status of fish populations exposed to sub-lethal pesticides concentrations. Changes in enzyme activity, oxidative stress markers, and metabolic functions indicate environmental stress, aiding bio-monitoring protocols for freshwater ecosystems (Mansour et al., 2021). The results of this study could inform regulatory frameworks and agricultural practices regarding pesticide application. By providing l data on Paraquat toxicity, the study may influence policies to mitigate agricultural runoff impacts on aquatic environments (Carpenter et al., 2021), leading to sustainable agricultural practices that protect freshwater resources. The outcomes of this research can pave the way for further studies exploring the chronic effects of Paraquat and similar chemicals on aquatic organisms. Understanding biochemical responses in C. gariepinus can help develop bio-monitoring protocols for assessing pollution in freshwater systems (Mansour et al., 2021). Furthermore, the findings may support the formulation of regulatory policies to curb herbicide runoff and promote sustainable agricultural practices (Sánchez et al., 2020; Carpenter et al., 2021). Therefore, this research contributes to the growing body of evidence on sub-lethal pesticide toxicity in fish providing essential data to inform policy, conservation efforts, and future research in aquatic toxicology.

Objectives of the Study

The objectives of this study are to:

1. Assess the sub-lethal effects of Paraquat dichloride (200 g/L) on biochemical parameters of juvenile Clarias gariepinus
2. Determine the mortality rates of juvenile Clarias gariepinus exposed to varying concentrations of Paraquat dichloride (200 g/L).

MATERIALS AND METHODS

Experimental Design

A controlled laboratory experiment was conducted to assess the sub-lethal effects of Paraquat dichloride on juvenile C. gariepinus. Five treatment groups (T1-T5) were established, with each group containing an equal number of juvenile C. gariepinus nine fish per replicate), with three replicates per group (n=135 total). This design allows for a comprehensive assessment of the biochemical dose-dependent responses to Paraquat exposure while minimizing variability within groups (Olufemi et al., 2021). Juvenile C. gariepinus were selected for the study due to their heightened sensitivity to environmental changes compared to adult fish. A 96-hour range-finding was conducted using ten 25L glass tanks capacity, each filled with 15 liters of water before the introduction of five (5) Clarias gariepinus juveniles for each treatment. The concentration values were 0.01ml/L, 0.05ml/L, 0.10ml/L, 0.15ml/L and 0.20ml/L. Each of the five varying concentrations was duplicated (totaling fifty (50) fish).

For the sub-lethal toxicity test, healthy fish were randomly selected, weighed and distributed into five (5) plastic aquaria (42.5 × 30.5 × 22.5cm) containing 50 liters of dechlorinated water.

Sample Collection

Juveniles of C. gariepinus were procured from the Teaching and Research farm of the Federal Polytechnic, Ado Ekiti, Ekiti State, Nigeria while Paraquat dichloride (200 g/L) was purchased as a commercially available herbicide from an agrochemical outlet in Ado Ekiti.

Experimental Procedure

Fish was acclimatized for two weeks in aerated, dechlorinated water under optimal water quality conditions.. During acclimatization, fish were fed with a commercial feed (42% protein) at 5% body weight, administered in two daily rations (7:00hrs and 17:00hrs) (Blažek et al., 2020). Feeding was stopped 24 hours prior to the commencement of the sub-lethal experiment. Fish in the experimental treatment group were exposed to respective Paraquat dichloride (200 g/L) for 28 days. The control group was maintained under identical conditions without Paraquat exposure, allowing for comparative analysis of biochemical parameters. Paraquat dichloride was administered at concentrations of 0.00ml/L (T1; control), 0.034ml/L (T2), 0.067ml/L (T3), 0.100ml/L (T4) and 0.134ml//L (T5) which are 0%, 16.67%, 33.33%, 50.00%, 66.67% of 0.2ml/L obtained as LC_{50}-96. Mortality checks were conducted twice daily, and fish were declared dead when there was no opercula movement and no response to gentle prodding. Dead fish were promptly removed using plastic forceps to maintain water quality (Adebayo et al., 2020).

Biochemical Analyses

After the exposure period, the fish were euthanized and blood samples were collected from the caudal vein using sterile syringes (Olufemi et al., 2021). The blood samples were dispensed into tubes containing ethylenediaminetetraacetate (EDTA) anticoagulant and transported in ice-packed bags to the Microbiological Laboratory unit of Ekiti State University Teaching Hospital, Ado Ekiti, for hematological analysis. Biochemical indices were analyzed according to standard analytical procedures described by Adebayo et al. (2020). These included total protein, albumin, globulin and blood glucose, which were estimated by means of spectrophotometric methods while Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated according to standard enzymatic methods. Levels of sodium, potassium, and calcium were analyzed using an electrolyte analyzer, with measurements conducted in triplicate to ensure reliability (Olufemi et al., 2021).

Statistical analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 20. ANOVA was employed to assess differences in biochemical parameters between the treatment and control groups. Post-hoc comparisons were conducted using Duncan's Multiple Range Test, with significance accepted at $p < 0.05$.

RESULTS AND DISCUSSION

Lengths and Weight of Juvenile Catfish exposed to Paraquat dichloride (200 g/L) for 28 days

Table 1 presents the initial and final weights and lengths of Clarias gariepinus exposed to various concentrations of Paraquat dichloride (200 g/L) . The initial weights and lengths of C. gariepinus across all treatment groups (T1 to T5) showed no significant differences, as indicated by the overlapping means with similar superscripts in each parameter. The initial weights ranged from 28.51g (T2) to 29.96g (T1), suggesting that the fish maintained relatively consistent initial body mass across the different treatments, while the initial standard lengths varied slightly, with T1 measuring 5.74 cm and T2 measuring 5.62 cm, again indicating no significant differences among the treatment groups. Similarly, the initial total lengths were consistent across treatments, with values ranging from 6.61 cm in T2 to 6.72 cm in T5. The final measurements also did not show statistically significant differences among treatments ($p > 0.05$), suggesting that sub-lethal exposure to Paraquat did not cause significant growth inhibition during the exposure period.

Table 1: Lengths and Weight of Juvenile Catfish Exposed to Paraquat dichloride (200 g/L) for 28 Days

Parameter	T1	T2	T3	T4	T5
Initial Weight (g)	29.96±8.19[a]	28.51±6.19[a]	29.31±9.24[a]	29.75±9.59[a]	29.91±6.28[a]

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Initial Standard length (cm)	5.74±0.52^{a}	5.62±0.51^{a}	5.79±0.49^{a}	5.82±0.69^{a}	5.78±0.50^{a}
Initial Total length (cm)	6.66±0.52^{a}	6.61±0.50^{a}	6.66±0.46^{a}	6.71±0.71^{a}	6.72±0.51^{a}
Final Weight (g)	29.14±9.80^{a}	26.85±7.09^{a}	27.08±6.16^{a}	31.00±8.75^{a}	27.17±3.31^{a}
Final Standard length (cm)	5.79±0.70^{a}	5.77±0.44^{a}	5.67±0.49^{a}	5.67±0.71^{a}	5.83±0.41^{a}
Final Total length (cm)	6.93±0.73^{a}	6.62±0.51^{a}	6.75±0.62^{a}	6.78±0.83^{a}	6.67±0.52^{a}

Means±SD on the same row with homogenous superscripts indicate no significant difference (p>0.05).

Mortality Rates of C. gariepinus exposed to varying concentration of Paraquat dichloride (200 g/L)

The mortality rates of Clarias gariepinus exposed to increasing concentrations of Paraquat dichloride (200 g/L) are shown in Table 2. The control group (T1) exhibited no mortality, indicating normal physiological function without the influence of the herbicide. In contrast, treatment groups T2 and T3 showed mortality rates of 27.78%, reflecting initial physiological stress without substantial lethality.

The mortality rates increased notably in T4 (50.00%) and T5 (66.67%), indicating a clear dose-dependent effect of Paraquat on fish mortality. The variation in mortality rates was statistically significant, with groups T4 and T5 demonstrating a higher likelihood of mortality compared to the lower concentration groups (T2 and T3). These findings demonstrate the detrimental impact of Paraquat dichloride on the biochemical health of C. gariepinus, with elevated mortality suggesting severe disruptions in metabolic and physiological processes linked to the herbicide's toxic effects.

Table 2: Mortality Rates of C. gariepinus exposed to varying concentration of Paraquat dichloride (200 g/L)

T1	T2	T3	T4	T5
0.00±0.00^{a}	27.78±7.86^{b}	27.78±7.86^{b}	50.00±7.86^{bc}	66.67±15.71^{c}

Means±SD on the same row with homogenous superscripts indicate no significant difference (p>0.05).

Biochemical Properties of C. gariepinus exposed to varying concentrations of Paraquat dichloride (200 g/L)

Table 3 summarizes the biochemical properties of C. gariepinus responses to varying Paraquat dichloride concentrations (200 g/L) for 28 days The results revealed the significant changes in glucose, cholesterol, liver enzymes (AST, ALT, ALP), indicating toxic effects of the herbicide. The glucose levels in the control group (T1) had the lowest of 0.95 ± 0.07 mmol/L. A significant increase in glucose levels, with the highest recorded in T3 (9.05 ± 0.07 mmol/L), moderately increase in T2 (6.90 ± 0.14 mmol/L) and T4 (6.95 ± 0.07 mmol/L), while T5 had a decrease to 3.70 ± 0.14 mmol/L. This increase in glucose, especially in T2, T3, and T4 indicate stress-induced hyperglycemia, as a result of Paraquat's toxic effects on the fish. The Cholesterol level was high in T4 (10.90 ± 0.14 mmol/L), followed by T1 (9.75 ± 0.07 mmol/L), indicating a potential increase in lipid metabolism or altered fat storage due to Paraquat exposure. In T2 (6.32 ± 0.01 mmol/L), the cholesterol levels were significantly lower, while T5 showed a moderate decrease (8.28 ± 0.31 mmol/L). The rise in cholesterol in T4 could be as a result of liver dysfunction due to Paraquat-induced oxidative stress.

AST levels in T1 (173.60 ± 0.57 IU/L) were significantly higher compared to T2 (96.80 ± 0.28 IU/L). The highest AST level was observed in T4 (195.65 ± 0.50 IU/L), indicating liver damage or injury due to Paraquat exposure. T3 (156.00 ± 1.41 IU/L) and T5 (151.57 ± 0.02 IU/L) showed intermediate increases, further supporting the observation that Paraquat disrupts liver function, leading to the release of AST into the bloodstream.ALT levels were highest in T5 (86.38 ± 0.88 IU/L) and T4 (85.88 ± 0.17 IU/L), both of which showed significantly elevated values compared to T1 (77.64 ± 0.51 IU/L). The ALT levels in T2 (40.84 ± 0.23

IU/L) had the lowest, suggesting minimal liver damage in this group. The increase in ALT levels in the higher exposure groups (T4 and T5) points to liver damage caused by Paraquat toxicity. However, a marked decrease in ALP levels of T4 (2.88 ± 0.17 IU/L), which could signify suppressed enzyme activity due to extensive liver damage or bone activity. The highest ALP activity was recorded in T2 (155.97 ± 0.04 IU/L), indicating significant liver dysfunction in this group. T1 (5.76 ± 0.34 IU/L), T3 (8.64 ± 0.51 IU/L), and T5 (11.02 ± 0.03 IU/L) exhibited moderate increases, revealing that Paraquat exposure induces some level of hepatic damage, although the effects were less pronounced in these groups compared to T2.

Table 3: Biochemical Properties of C. gariepinus exposed to varying concentrations of Paraquat dichloride (200 g/L)

Treatment	T1	T2	T3	T4	T5
GLUCOSE (mmol/L)	$0.95{\pm}0.07^{a}$	$6.90{\pm}0.14^{c}$	$9.05{\pm}0.07^{d}$	$6.95{\pm}0.07^{c}$	$3.70{\pm}0.14^{b}$
CHOLESTEROL (mmol/L)	$9.75{\pm}0.07^{d}$	$6.32{\pm}0.01^{a}$	$7.37{\pm}0.02^{b}$	$10.90{\pm}0.14^{e}$	$8.28{\pm}0.31^{c}$
AST(IU/L)	$173.60{\pm}0.57^{d}$	$96.80{\pm}0.28^{a}$	$156.00{\pm}1.41^{c}$	$195.65{\pm}0.50^{e}$	$151.57{\pm}0.02^{b}$
ALT(IU/L)	$77.64{\pm}0.51^{c}$	$40.84{\pm}0.23^{a}$	$63.68{\pm}0.45^{b}$	$85.88{\pm}0.17^{d}$	$86.38{\pm}0.88^{d}$
ALP (IU/L)	$5.76{\pm}0.34^{b}$	$155.97{\pm}0.04^{e}$	$8.64{\pm}0.51^{c}$	$2.88{\pm}0.17^{a}$	$11.02{\pm}0.03^{d}$

Means±SD on the same row with homogenous superscripts indicate no significant difference (p>0.05).

The increasing mortality rates of Clarias gariepinus exposed to varying concentrations of Paraquat dichloride underscore the critical need to understand the biochemical responses of fish to such environmental stressors. The results of this study reveal a dose-dependent relationship between Paraquat exposure and mortality in Clarias gariepinus, underscoring the herbicide's detrimental impact on fish physiology. . The biochemical composition of fish, particularly their enzyme systems and metabolic pathways, plays a vital role in their ability to cope with such stressors. As observed in this study (Table 2), the control group (T1) displayed no mortality, which indicates a baseline for healthy physiological functioning. However, the significant increase in mortality rates were observed in the exposed groups (T2 to T5), indicating that as the concentration of Paraquat increased, the fish's ability to maintain homeostasis deteriorated (Khan et al., 2021).

Paraquat dichloride is known to generate reactive oxygen species (ROS), which overwhelm the fish's antioxidant defenses and induce oxidative stress (Ameen et al., 2018). In this study, the significant increase in mortality, as observed in treatment groups T4 (50.00%) and T5 (66.67%).The heightened oxidative stress likely disrupted normal cellular function in the liver and other vital organs, leading to systemic failure. Biochemical markers such as superoxide dismutase (SOD) and catalase play vital roles in neutralizing ROS and are critical in maintaining cellular integrity (Khan et al., 2021). However, prolonged Paraquat exposure may have suppressed their activity, thus increasing the susceptibility of fish to oxidative damage and death. Research has shown that prolonged exposure to toxic substances can deplete antioxidant reserves and promote lipid peroxidation, protein oxidation, and ultimately resulting in cell death (Matsuo et al., 2015). This oxidative damage can directly impact hematological parameters, and exacerbating the effects seen in our mortality data, where impaired blood parameters may lead to reduced oxygen transport could have amplified physiological stress (Blažek et al., 2020). The mortality rates observed in this study under stressed fish tend to shift toward energy metabolism, often favoring anaerobic pathways due to compromised aerobic metabolism (Adebayo et al., 2020). This shift can lead to increased lactic acid build up and depletion of glycogen stores, further stressing the organism and contributing to mortality.

The biochemical consequences of Paraquat exposure also include alterations in lipid metabolism. Disruptions in lipid profiles can have cascading effects on fish growth, reproduction, and survival, as indicated by the high mortality rates in T4 and T5. The implications of these biochemical responses extend beyond individual fish to broader ecological impacts (García et al., 2022). However, prolonged Paraquat exposure may have suppressed their activity, thus increasing the susceptibility of fish to oxidative damage and death. These biochemical stressors increase the mortality rate which can disrupt aquatic food webs and decline in fish population (Baker et al., 2019). The high mortality rates observed indicated the need for monitoring and regulation of pesticide in aquatic ecosystems.

The findings in this study suggest that exposure of C. gariepinus (African catfish) to varying concentrations of Paraquat dichloride, a widely used herbicide, and leads to significant changes in several biochemical markers related to fish health. The biochemical responses further support the observed physiological stress. The significant elevation in glucose levels in treatments (T2, T3, and T4) with higher Paraquat exposure due to a typical stress response in fish. . This is likely a result of Paraquat induced metabolic disruption triggering the mobilization of glucose levels, as the body mobilizes energy reserves to cope with environmental stressors (Davis et al., 2018), to meet increased demands under oxidative stress in C. gariepinus. This could impair normal metabolic function, leading to long-term health issues such as insulin resistance or metabolic disorders (Little et al., 2003).

The higher cholesterol levels in T4 (10.90 ± 0.14 mmol/L) and moderate high in T3 and T5 suggest disruptions in the lipid metabolism and liver function. Elevated cholesterol accumulation levels in fish due to Paraquat exposure alteration imply hepatic dysfunction due to oxidative stress (Zhou et al., 2011). . On the other hand, the decreased observed in T2, with lower cholesterol indicate impaired or altered lipid processing due to early-stage toxicity.

Liver enzyme activities further confirm the hepatotoxic effects of Paraquat. The significant elevations in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in T4 and T5 suggest severe liver cell damage. These enzymes are reliable biomarkers for hepatocellular injury as they leak into the bloodstream upon damage to liver tissues (Ibrahim & Dawood, 2018). These finding indicate that that C. gariepinus may experience significant liver damage at higher concentrations of Paraquat, which could impair the fish's overall health, immune function, and growth.

Similarly, the significantly elevated ALP levels in T2 (155.97 ± 0.04 IU/L) suggest possible effects on the liver, bones, or other tissues responsible for alkaline phosphatase production while its suppression in T4 may be indicative of enzyme inhibition or severe liver dysfunction or change in metabolic processes (Zhou et al., 2011). The findings indicate that exposure to Paraquat dichloride disrupts normal physiological functions in C. gariepinus.

Collectively, the biochemical alterations observed in glucose, cholesterol, AST, ALT, and ALP levels point demonstrate that exposure to Paraquat compromises multiple physiological systems in C. gariepinus. These effects are consistent with hepatotoxicity, metabolic dysregulation, and oxidative stress, which collectively impair fish health and contribute to increased mortality. Chronic exposure to such pollutants may result in sub-lethal effects that reduce fitness, growth, reproductive success, and resistance to disease (Moniruzzaman et al., 2020).

From the ecological perspective, the implications of these findings are profound. Elevated mortality and sub-lethal biochemical stress in fish can affect the entire aquatic food webs, affecting biodiversity and ecosystem stability. Fish like C. gariepinus, serve as critical bio-indicators in ecotoxicology studies, can provide valuable insights into the health of aquatic ecosystems under chemical stress (Boudou & Ribeyre, 2006). The study highlights the urgent need for stringent regulation and monitoring of pesticides and herbicides usage to mitigate the adverse impacts of agricultural runoff on aquatic life (Van Dyk et al., 2020).

CONCLUSION

This study establishes that the biochemical properties of fish are vital indicators of health and responses to environmental stressors. The observed mortality rates of Clarias gariepinus exposed to Paraquat dichloride reflect significant biochemical disturbances associated with oxidative stress and metabolic disruption. These changes are indicative of oxidative stress, liver dysfunction, and metabolic disruption, which can have serious implications for fish health.

The findings emphasize the need for an effective regulation and continuous monitoring of chemical pollutants like Paraquat is essential for maintaining healthy aquatic ecosystems and the biodiversity they support. Understanding these biochemical responses for assessing the ecological impact of pollutants and implementing effective conservation strategies is crucial. Future research should focus on comprehensive assessments of biochemical markers in various fish species to enhance the understanding of aquatic toxicology to inform more robust conservation and management strategies. In addition, to encourage the understanding of specific biochemical pathways affected by Paraquat and similar pollutants in fish will enhance management practices and improve knowledge that can mitigate the toxic effects of toxic substances in aquatic environments

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