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**Research Article**

# Investigating the Impacts of Hatchery Application of Chlorine, Hydrogen Peroxide, Sodium Chloride on Survival and Haematological Profiles of *Clarias gariepinus* Juveniles

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**Abstract**

Indiscriminate use of disinfectants in aquaculture caused ecological problems to aquatic organisms. This study was aimed to assess the median lethal concentrations 96h-LC50 of Chlorine, Hydrogen peroxide, and Sodium chloride on survival and hematological parameters of *Clarias gariepinus* juveniles. Three hundred and sixty fish (n=360) was divided into three groups, 120 fish (n= 120/ per disinfectant), each 120 fish was divided into five groups (n= 24/group). The fish were exposed to Chlorine (3mg, 9mg, 15mg, 21mg L-1), hydrogen peroxide (3mg, 9mg, 15mg, 21mg L-1), sodium chloride (3g, 9g, 15g, 21g L-1) and a control group for 96 hours. The median lethal concentration 96h-LC50 was evaluated using Probit analysis IBM SPSS 23 and haematological parameters were evaluated using one way ANOVA, significance is considered as $p \leq 0.05$. The 96-LC50 values were: 7.422mgL-1 (Chlorine), 9.075mgL-1 (Hydrogen peroxide), and 10.172gL-1 (Sodium chloride). Sodium chloride, hydrogen peroxide and chlorine showed highest, moderate and lowest survival rates, respectively. Significant ($p<0.05$) anaemia, leukocytosis, and heterophilia were observed in groups exposed to chlorine, hydrogen peroxide (3 mg/L – 21mg/L) and Sodium chloride (3 g/L – 21g/L) compared to control group. Reduced hemoglobin concentration and red blood cell counts were observed in fish exposed to chlorine and hydrogen peroxide (15 mg/L – 21mg/L) compared to control group. In this study, exposure to low and high concentrations of chlorine, hydrogen peroxide, and sodium chloride induced stress and altered the hematological parameters in *Clarias gariepinus* juveniles.

Keywords: Chlorine, disinfectants, hematology, hydrogen peroxide, sodium chloride

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## Introduction

Aquaculture is a faster growing sector of food production in the world. The cultivation of aquatic animals in a controlled environment is called aquaculture. The controlled environments could be freshwater and saltwater depending on the geographical location of the environment. Aquaculture provides a sustainable, alternative, cheaper, and abundant protein source to reduce global food insufficiency (Bhujel 2024).

Moreover, varying dietary habits and increased population expanded aquaculture from sustenance to large-scale production (Saini et al 2024). In Nigeria, the phenomenal growth in aquaculture industry is majorly due to large production of African catfish, *Clarias gariepinus (C. gariepinus)*, a member of the family Clariidae (Olagunju 2024).

The popular production of *C. gariepinus* in Nigeria is attributed to high growth rate, and palatability. In addition, other qualities included resilience, ability to endure a wide range of temperatures, low dissolved oxygen, and salinity (Remilekun et al 2021). Reproduction of *C. gariepinus* does not occur in captivity. Meanwhile, artificial propagation provided means of seedlings through possibility of controlled spawning and hatching. Despite this potential, the aquaculture industry faces significant impediments, primarily due to diseases and improper management, which lead to substantial economic losses (Anifowose et al 2024a).

*C. gariepinus* is susceptible to bacterial and parasitic infections. Several factors are contributed to the susceptibility, these include poor water quality such

as inadequate water exchange, buildup of organic matter, excessive ammonia levels, and stressful conditions which might be overcrowding, handling, and transportation (Latief et al 2024). The stressors can drastically affect the immune system and increase the vulnerability of fish to disease. The mortality rate due to infectious diseases is high with huge economic loss in aquaculture compared to terrestrial livestock (Haenen et al 2023).

Furthermore, the environmental impacts of aquaculture including nutrient runoff, require careful management to mitigate negative effects on surrounding ecosystems.

Disinfectants are chemical compounds used in aquaculture to control fish pathogens that can cause disease in aquatic animals. The chemical substances are used for years to protect fish from diseases (Dinesh et al 2022). The disinfectants commonly used included chlorine, formalin, hydrogen peroxide, potassium permanganate, and sodium chloride. Nevertheless, indiscriminate use of these chemicals, application of excessive amounts, and stocking fish immediately after usage, can damage the aquatic environment (Yeşilbudak 2024). Moreover, the widespread usage, especially in developing countries, has raised concerns about potential ecotoxicity. Excessive application of disinfectant can adversely impact fish survival, haematology, and histopathology, leading to economic and fish seed loss. The critical factors for successful aquaculture management are concentration of disinfectants and the timing on fish exposure (Newman 2021).

Blood analysis is a crucial tool for assessing the toxic effects of chemicals on aquatic organisms (Bojarski et al 2025). Regular clinical blood examination is an important tool for diagnosing health challenges (Ekici et al 2021). Hematological parameters can reveal physiological changes long before visible symptoms appear. The red and white blood cells, hemoglobin content, and hematocrit values in fish can be changed as a result of exposure to excessive disinfectants. In addition, stress responses can be initiated through exposure of fish to high level of disinfectants. This stress load may compromise physiological and biochemical functions, resulting into detrimental health effects (Witeska et al 2023). The damage of DNA and formation of nuclear abnormalities caused by abnormal usage of disinfectant can be evaluated through presence of micronuclei in in fish erythrocytes. The stress could be assessed through changes in common haematological variables, haemoglobin content, and hematocrit values. Previous studies have shown that *C. gariepinus* exposed to disinfectants exhibited significant alterations in the blood parameters (Xu et al 2021). The damage of DNA and production of nuclear abnormalities in erythrocytes are caused by excessive concentration of disinfectants at the cellular level. Therefore, the occurrence of micronuclei in fish erythrocytes provided a valuable biomarker for assessing genotoxicity in aquatic environments during in vivo laboratory studies (D'Agostini et al 2021).

The younger fish showed more symptoms of negative impact of excessive concentration of disinfectants. In addition, some disinfectants can bioaccumulate in fish tissue, posing risks to species higher up the food chain. Constant exposure to sub-lethal disinfectant concentrations led to physiological and behavioral changes that may reduce fish populations and decrease predator avoidance.

However, this study is aimed to investigate the toxicological responses of the African catfish, *C. gariepinus*, to exposure chlorine, hydrogen peroxide, and sodium chloride by immersion of water in fish hatchery.

## Material and Methods

### Ethics approval and consent to participate

Handling of experimental Fish, Ethical clearance, and valid approval were obtained from the Animal Care and Use Research and Ethics Committee (ACUREC) of the University of Ibadan in order to use animals for our studies on disinfectants (NHREC/UIACUREC/05/12/2022A).

### Experimental animals and acclimatization

Three hundred and sixty juveniles of Clarias gariepinus (n = 360 with an average weight of 3.47±0.95 g, length of 6 ± 0.7 cm was procured from commercial fish farm in Ibadan. The fish were stored in 1000 L plastic tanks and acclimatized for 2 weeks. During the acclimatization period, the fishes were fed with commercial feed twice daily ad libitum and the stock water was changed once every other day to prevent the accumulation of decaying food particles and waste metabolites.

### Acute toxicity

The fish acute toxicity test employed in this study is TG203 and OECD (2019). Chlorine® (USA), Hydrogen peroxide®, (Nigeria), and Sodium chloride®, (China) were purchased for acute toxicity tests on *Clarias gariepinus* juveniles. Acute toxicity test of the chemicals was carried out to determine the lethal concentrations 96h- $LC_{50}$ to *C. gariepinus*. The *C. gariepinus* juveniles were subjected to each chemical toxicity test for 96 hours, to determine the toxic concentration range and the related timing OECD (2019). The test was carried out using 10L plastic tanks and the fish were distributed equally into five different

Table 1. Toxicity study (96h-$LC_{50}$) for Chlorine on *Clarias gariepinus* Juvenile

| Concentration of Chlorine (mg/L) | Log of Conc. | Number of Subjects | Observed Responses | Expected Responses | Residual | Probability |
|---|---|---|---|---|---|---|
| 0 | 0.000 | 24 | 0 | 0.000 | 0.000 | .000 |
| 3 | 0.477 | 24 | 8 | 6.617 | 1.383 | .276 |
| 9 | 0.954 | 24 | 10 | 13.210 | -3.210 | .550 |
| 15 | 1.176 | 24 | 16 | 16.276 | -276 | .678 |
| 21 | 1.322 | 24 | 20 | 18.071 | 1.020 | .752 |
| Probability | Chlorine (mg/L) | | | | | |
| | 96h-$LC_{50}$ | Lower Limit | Upper Limit | | | |
| 0.05 | 7.422 | 3.989 | 11.195 | | | |

groups; 120 fish were divided into five groups (n= 24). The groups were; A, B, C, D, and E. Pilot study was earlier carried using varying concentration of chlorine, hydrogen peroxides, and sodium chloride on *Clarias gariepinus* juveniles. The first group was control, while, varying concentrations of Chlorine was added to the water at 3mg/L, 9mg/L, 15mg/L, 21mg/L, respectively. Every 12 hours, mortality rates were monitored and recorded. The 96h-$LC_{50}$ was calculated using Probit Analysis method (Uçar 2024). Another 120 fish (n = 120) were divided into five groups and 96h-$LC_{50}$ acute toxicity test was carried out using hydrogen peroxide with varying concentrations was added to the water at 3mg/L, 9mg/L, 15mg/L, 21mg/L, respectively. Moreover, the last 120 fish (n = 120) was divided into five groups and Sodium chloride with varying concentrations was added to water at 3g/L, 9g/L, 15g/L, and 21g/L, respectively (Pulido-Reyes et al 2024). The fish were subjected to photoperiod of 12 hours light (daylight) and 12 hours darkness (midnight). The water in the plastic tank was not changed throughout 96 hours.

Fifty millilitres of water samples in the plastic tanks were collected aseptically using sterile cap bottles. The water samples were transported to Aquatic Animal and Wildlife Laboratory, Department of Veterinary medicine, University of Ibadan. For water quality parameters, namely; Dissolved Oxygen (DO), pH, Nitrite, Alkalinity, and Ammonia were determined according to APHA (1995). The parameters like DO and pH were determined directly in the ponds using YSI 556-meter function as pH and DO meter, temperature with a Hach conductivity meter (Model DR2400). Ammonia and nitrite were determined by chemical method and ELISA reader using optical density of color development at different wavelength.

## Hematology

Following 96 hours of acute toxicity, four fish (n=4) were randomly sampled from each tank to collect blood. Each of the sampled fish was handled with physical restrain and manual covering of eyes with a sterile glove. For each sampled fish, blood was drawn from the caudal vein using a 1-mL sterile hypodermal syringe with a 24-gauge needle. The collected blood was then transferred into heparinized Eppendorf tubes and transported to the Clinical Laboratory of the Department of Veterinary Medicine, University of Ibadan. A haematological analysis was subsequently performed on the samples according to the method described by Adeshina et al (2020).

Table 2. Toxicity study (96h-$LC_{50}$) for Hydrogen peroxide on *Clarias gariepinus* Juvenile

| Concentration of Hydrogen peroxides (mg/L) | Log of Conc. | Number of Subjects | Observed Responses | Expected Responses | Residual | Probability |
|---|---|---|---|---|---|---|
| 0 | 0.000 | 24 | 0 | 0.000 | 0.000 | .000 |
| 3 | 0.477 | 24 | 8 | 7.296 | .704 | .304 |
| 9 | 0.954 | 24 | 10 | 11.221 | -1.221 | .468 |
| 15 | 1.176 | 24 | 12 | 13.138 | -1.138 | .547 |
| 21 | 1.322 | 24 | 16 | 14.381 | 1.619 | .599 |
| Probit Analysis | | | | | | |
| Fish Type | Probability | Hydrogen peroxide (mg/L) | | | | |
| | | 96h-$LC_{50}$ | Lower Limit | Upper Limit | | |
| Juvenile | 0.05 | 9.075 | 3.858 | 86.804 | | |

| Table 3. Toxicity study (96h-$LC_{50}$) for Sodium chloride on *Clarias gariepinus* Juvenile | | | | | | |
|---|---|---|---|---|---|---|
| Concentration of Sodium chloride (g/L) | Log of Conc. | Number of Subjects | Observed Responses | Expected Responses | Residual | Probability |
| 0 | 0.000 | 24 | 0 | 0.000 | 0.000 | .000 |
| 3 | 0.477 | 24 | 0 | .000 | .000 | .000 |
| 9 | 0.954 | 24 | 0 | .019 | -.019 | .001 |
| 15 | 1.176 | 24 | 1 | 2.759 | -1.759 | .120 |
| 21 | 1.322 | 24 | 14 | 12.620 | 1.380 | .549 |
| Probit Analysis | | | | | | |
| Fish Type | Probability | Sodium chloride (g/L) | | | | |
| | | 96h-$LC_{50}$ | Lower Limit | Upper Limit | | |
| Juvenile | 0.05 | 10.172 | 9.136 | 12.679 | | |

### Statistical analysis

The tests for normality and homogeneity of variance were performed on the data obtained. Statistical software SPSS version 23 was employed for statistical analysis of data. The Probit Analysis method as reported by Uçar (2024) was used to estimate the lethal concentration value of 96-h $LC_{50}$. Analysis of variance (ANOVA) and Duncan multiple range test (DMRT) were employed to calculate the level of significance observed in haematological parameters Differences were considered significant at $p \leq 0.05$ for all the datasets. Superscripts in the tables are used to indicate significant differences.

## Results

The behavior of the fish, *C. gariepinus*, changed as they were exposed to disinfectants. As the concentration and exposure time of the disinfectant increased, the fish showed more abnormal behaviors like hyperactivity, erratic swimming, and loss of equilibrium. While somersaulting and jumping were observed at higher concentrations, the activities ceased at lower concentrations of the disinfectant. The exposed fish demonstrated a reduction in movement, including a slower swimming rate, minimal fin movement, and eventually settling motionless at the bottom of the tank. No fish in the control group died during the exposure period. The lethal concentrations for chlorine, hydrogen peroxide, and sodium chloride were determined using IBM SPSS 23 Probit Analysis, with results presented in Tables 1, 2, and 3, respectively. The experimental 96h-LC50 for chlorine was lowest in *C. gariepinus* which was 7.422mg/L indicating most toxic. Hydrogen peroxide 9.075mg/L was lower in *C. gariepinus* indicating moderate toxicity, while, sodium chloride was highest in C. gariepinus was 10.172g/L indicating the least toxic disinfectant.

The survival rate was decreasing with an increase in concentration of chlorine exposed to *C. gariepinus*. The survival rate was decreasing with an increase in concentration of hydrogen peroxide to *C. gariepinus*, meanwhile, survival rate remained the same with an increase in concentration of sodium chloride exposed to *C. gariepinus*. Although, 21 g/L showed lowest survival rate of 10. Sodium chloride showed highest survival rate compared to other disinfectants, meanwhile, hydrogen peroxide indicated a moderate survival rate and chlorine showed lowest survival rate (Table 4). Significant ($p<0.05$) anaemia, leukocytosis, and heterophilia were observed in groups exposed to chlorine (3 mg/L – 21mg/L) compared

| Table 4.Survival Rate of *Clarias gariepinus* juveniles exposed to varying concentrations of Chlorine, Hydrogen peroxide and Salt for 96 hours | | | | | |
|---|---|---|---|---|---|
| Concentration of Chlorine mg/L | Survival rate | Concentration of Hydrogen peroxide mg/L | Survival rate | Concentration of Sodium chloride g/L | Survival rate |
| 0 | 24 | 0 | 24 | 0 | 24 |
| 3 | 16 | 3 | 16 | 3 | 24 |
| 9 | 14 | 9 | 14 | 9 | 24 |
| 15 | 8 | 15 | 12 | 15 | 23 |
| 21 | 4 | 21 | 8 | 21 | 10 |

Table 5.Haematology of *Clarias gariepinus* juveniles exposed to Chlorine at varying concentration for 96 hours

| Parameters | Control (M±SEM) | Chlorine 3mg/L (M±SEM) | Chlorine 9mg/L (M±SEM) | Chlorine 15mg/L (M±SEM) | Chlorine 21mg/L (M±SEM) |
|---|---|---|---|---|---|
| PCV (%) | 21.33±0.67$^{c}$ | 19.33±0.33$^{b}$ | 18.00±0.58$^{b}$ | 17.67±1.67$^{b}$ | 16.00±0.31$^{a}$ |
| Hb (%) | 7.00±1.00$^{c}$ | 7.83±0.12$^{c}$ | 7.07±0.27$^{c}$ | 6.23±0.53$^{b}$ | 4.83±0.23$^{a}$ |
| RBC (x $10^{12}$/L) | 6.17±0.17$^{c}$ | 6.71±0.14$^{c}$ | 5.55±0.07$^{b}$ | 5.00±0.22$^{b}$ | 4.71±0.45$^{a}$ |
| WBC (x $10^{9}$/L) | 3.60±0.23$^{a}$ | 4.87±0.13$^{b}$ | 4.80±0.12$^{b}$ | 4.61±0.20$^{b}$ | 4.87±0.58$^{b}$ |
| Lymphocytes (%) | 40.00±1.15$^{b}$ | 39.00±0.58$^{a}$ | 37.33±0.88$^{a}$ | 38.33±0.67$^{a}$ | 44.00±0.60$^{c}$ |
| Heterophils (%) | 59.00±1.15$^{b}$ | 61.60±1.15$^{c}$ | 62.33±0.67$^{c}$ | 61.33±2.33$^{c}$ | 55.70±0.067$^{a}$ |
| Monocytes (%) | 1.00±0.00$^{b}$ | 0.40±0.58$^{a}$ | 0.33±0.33$^{a}$ | 0.34±0.33$^{a}$ | 0.30±0.29$^{a}$ |

Values with different superscript along the row are statistically significant ($p < 0.05$) according to ANOVA

Table 6.Haematology of *Clarias gariepinus* juveniles exposed to Hydrogen peroxide at varying concentration for 96 hours

| Parameters | Control (M±SEM) | Hydrogen peroxide 3mg/L (M±SEM) | Hydrogen peroxide 9mg/L (M±SEM) | Hydrogen peroxide 15mg/L (M±SEM) | Hydrogen peroxide 21mg/L (M±SEM) |
|---|---|---|---|---|---|
| PCV (%) | 21.33±0.67$^{a}$ | 19.33±0.33$^{b}$ | 18.00±0.58$^{b}$ | 16.00±0.31$^{c}$ | 15.90±0.15$^{c}$ |
| Hb (%) | 7.00±1.00$^{a}$ | 5.83±0.12$^{b}$ | 5.07±0.27$^{b}$ | 4.83±0.23$^{c}$ | 4.07±0.32$^{c}$ |
| RBC (x $10^{12}$/L) | 6.17±0.17$^{a}$ | 6.71±0.14$^{a}$ | 5.55±0.07$^{b}$ | 4.71±0.45$^{b}$ | 4.55±0.06$^{b}$ |
| WBC (x $10^{9}$/L) | 3.60±0.23$^{a}$ | 4.87±0.13$^{b}$ | 4.80±0.12$^{b}$ | 6.87±0.58$^{c}$ | 6.80±0.21$^{c}$ |
| Lymphocytes (%) | 40.00±1.15$^{a}$ | 42.00±0.58$^{b}$ | 42.33±0.88$^{b}$ | 45.00±0.60$^{c}$ | 45.33±0.34$^{c}$ |
| Heterophils (%) | 59.00±1.15$^{a}$ | 57.00±1.15$^{b}$ | 56.33±0.67$^{b}$ | 54.00±0.067$^{c}$ | 54.63±0.47$^{c}$ |
| Monocytes (%) | 1.00±0.00$^{a}$ | 1.00±0.58$^{a}$ | 0.33±0.33$^{b}$ | 1.00±0.29$^{a}$ | 1.04±0.45$^{a}$ |

Values with different superscript along the row are statistically significant ($p < 0.05$) according to ANOVA

Table 7.Haematology of *Clarias gariepinus* juveniles exposed to Sodium chloride at varying concentration for 96 hours

| Parameters | Control (M±SEM) | Sodium chloride 3g/L (M±SEM) | Sodium chloride 9g/L (M±SEM) | Sodium chloride 15mg/L (M±SEM) | Sodium chloride 21mg/L (M±SEM) |
|---|---|---|---|---|---|
| PCV (%) | 21.33±0.67$^{a}$ | 20.67±1.67$^{a}$ | 0.00±0.58$^{a}$ | 20.00±0.31$^{a}$ | 18.13±1.43$^{b}$ |
| Hb (%) | 7.00±1.00$^{a}$ | 6.93±0.53$^{a}$ | 7.07±0.27$^{a}$ | 6.83±0.23$^{a}$ | 4.20±0.21$^{b}$ |
| RBC (x $10^{12}$/L) | 6.17±0.17$^{a}$ | 6.00±0.22$^{a}$ | 6.55±0.07$^{a}$ | 6.71±0.45$^{a}$ | 4.55±0.15$^{b}$ |
| WBC (x $10^{9}$/L) | 3.60±0.23$^{a}$ | 3.61±0.20$^{a}$ | 3.80±0.12$^{a}$ | 3.87±0.58$^{a}$ | 5.49±0.35$^{b}$ |
| Lymphocytes (%) | 40.00±1.15$^{a}$ | 40.33±0.67$^{a}$ | 41.33±0.88$^{a}$ | 40.00±0.60$^{a}$ | 42.10±0.58$^{b}$ |
| Heterophils (%) | 59.00±1.15$^{a}$ | 59.33±2.33$^{a}$ | 58.33±0.67$^{a}$ | 59.00±0.067$^{a}$ | 56.56±2.12$^{b}$ |
| Monocytes (%) | 1.00±0.00$^{a}$ | 0.34±0.33$^{a}$ | 0.34±0.33$^{a}$ | 1.00±0.29$^{a}$ | 1.34±0.26$^{b}$ |

Values with different superscript along the row are statistically significant ($p < 0.05$) according to ANOVA

to control group. Reduced haemoglobin concentration and red blood cell counts were observed in exposed to chlorine (15 mg/L – 21mg/L) compared to control group. Lymphopenia was observed in groups exposed to chlorine (3 mg/L – 15 mg/L) compared to control group, meanwhile, group exposed to chlorine 21 mg/L showed lymphocytosis compared to control group (Table 5).

Significant ($p<0.05$) anaemia, leukocytosis, and heteropenia were observed in groups exposed to hydrogen peroxide (3 mg/L – 21mg/L) compared to control group. Reduced haemoglobin concentration and red blood cell counts were observed in exposed to hydrogen peroxide (3 mg/L – 21mg/L) compared to control group. Lymphocytosis was observed in groups exposed to

Table 8.Physicochemical findings of experimental water tanks during exposure of *Clarias gariepinus* juveniles to Chlorine at varying concentration for 96 hours

| Parameters | Control (M±SEM) | 3g/L (M±SEM) | 9g/L (M±SEM) | 15mg/L (M±SEM) | 21mg/L (M±SEM) | Standard Values for Fish Farming APHA 1989, Boyd and Tucker 1992 |
|---|---|---|---|---|---|---|
| pH | 7.00±0.10[a] | 6.80±0.10[a] | 6.60±0.10[a] | 6.40±0.10[a] | 5.9±0.10[b] | 6.5 – 8.5 |
| Nitrite (ppm) | 0.00±0.00[b] | 0.01±0.008[b] | 0.01±0.008[b] | 0.01±0.008[b] | 0.01±0.008[b] | 0.05 max |
| Ammonia (ppm) | 0.00±0.00[b] | 0.10±0.02[b] | 0.10±0.02[b] | 0.30±0.02[b] | 0.50±0.02[b] | 2.0 max |
| Dissolved Oxygen (ppm) | 8.5±1.0[a] | 8.5±1.0[a] | 7.9±1.0[a] | 5.5±1.0[b] | 4.0±1.0[b] | 5.0 min |

Values with different superscript along the row are statistically significant ($p < 0.05$) according to ANOVA

Table 9.Physicochemical findings of experimental water tanks during exposure of *Clarias gariepinus* juveniles to Hydrogen peroxide at varying concentration for 96 hours

| Parameters | Control (M±SEM) | 3g/L (M±SEM) | 9g/L (M±SEM) | 15mg/L (M±SEM) | 21mg/L (M±SEM) | Standard Values for Fish Farming APHA 1989, Boyd and Tucker 1992 |
|---|---|---|---|---|---|---|
| pH | 7.00±0.10[a] | 6.6±0.10[a] | 6.5±0.10[a] | 6.3±0.10[a] | 5.9±0.10[b] | 6.5 – 8.5 |
| Nitrite (ppm) | 0.00±0.00[b] | 0.01±0.008[b] | 0.01±0.008[b] | 0.01±0.008[b] | 0.01±0.008[b] | 0.05 max |
| Ammonia (ppm) | 0.00±0.00[b] | 0.10±0.02[b] | 0.10±0.02[b] | 0.30±0.02[b] | 0.50±0.02[b] | 2.0 max |
| Dissolved Oxygen (ppm) | 8.5±1.0[a] | 8.5±1.0[a] | 6.8±1.0[b] | 5.0±1.0[b] | 4.5±1.0[b] | 5.0 min |

Values with different superscript along the row are statistically significant ($p < 0.05$) according to ANOVA

Table 10.Physicochemical findings of experimental water tanks during exposure of *Clarias gariepinus* juveniles to Sodium chloride at varying concentration for 96 hours

| Parameters | Control (M±SEM) | 3g/L (M±SEM) | 9g/L (M±SEM) | 15mg/L (M±SEM) | 21mg/L (M±SEM) | Standard Values for Fish Farming APHA 1989, Boyd and Tucker 1992 |
|---|---|---|---|---|---|---|
| pH | 7.00±0.10[a] | 7.0±0.10[b] | 7.0±0.10[b] | 7.0±0.10[b] | 7.0±0.10[b] | 6.5 – 8.5 |
| Nitrite (ppm) | 0.00±0.00[b] | 0.01±0.008[b] | 0.01±0.008[b] | 0.01±0.008[b] | 0.01±0.008[b] | 0.05 max |
| Ammonia (ppm) | 0.00±0.00[b] | 0.10±0.02[b] | 0.10±0.02[b] | 0.30±0.02[b] | 0.50±0.02[b] | 2.0 max |
| Dissolved Oxygen (ppm) | 8.5±1.0[a] | 8.5±1.0[a] | 8.5±1.0[a] | 8.5±1.0[a] | 6.7±1.0[b] | 5.0 min |

Values with different superscript along the row are statistically significant ($p < 0.05$) according to ANOVA

chlorine (3 mg/L – 21 mg/L) in a dose-dependent manner compared to control group (Table 6).

Significant ($p<0.05$) anaemia, leukocytosis, and heteropenia were observed in group exposed to Sodium chloride (21g/L) compared to control group. Reduced haemoglobin concentration and red blood cell counts were observed in group exposed to Sodium chloride (21g/L) compared to control group. Lymphocytosis was observed in groups exposed to Sodium chloride (21 g/L) in compared to control group (Table 7).

## Discussion

Disinfectants are chemicals used in aquaculture for control and prevention of infection. The effect of a particular disinfectant may not be targeted on specific pathogens. The results of this study in acute toxicity to the exposed catfish showed that the median lethal toxicity was 7.422 mg/L for chlorine, 9.075 mg/L for hydrogen peroxide, and 11.075 $gL^{-1}$ for sodium chloride. The result in this study showed that acute toxicity required higher mass volume in sodium chloride compared to other disinfectants. Meanwhile, sodium chloride caused least damage on haematology of the fish among the assessed disinfectants.

This difference in toxicity may be compared with their respective specific mechanism of action, varying degrees of toxicity, and chemical composition. Toxicity of chlorine disinfectants showed caused damage to respiratory and digestive system leading into mortalities (Kolawole and Iyiola 2021).

The aquatic usefulness of sodium chloride included, growth enhancement, parasitic treatment, and control of toxicity. In addition, sodium chloride was reported to show effective treatment of fresh water fish external parasitic infection and improve stress during transport (Tavares-Dias 2022). This corroborates that sodium chloride is least toxic. Though, the effectiveness of sodium chloride is related to species and concentration used (Tavares-Dias 2022). This implies that additional study on mode

of action of these disinfectants is necessary to elucidate responses from acute toxicity in this study.

Furthermore, responses of fish due to toxicity are related and dependent on factors such as characteristics of the chemical involved, environmental effect, susceptibility of the fish, disinfectant specificity, and aquatic animal species specificity.

For example, 96-h $LC_{50}$ value of 9.075 $mgL^{-1}$ observed for hydrogen peroxide in this present study was lower than the 10.172 g/L reported for sodium chloride but higher than that of chlorine (7.422 $mgL^{-1}$). Though hydrogen peroxide is much more toxic on a mass-unit basis which was indicated in sub-acute test on haematology of the fish at different concentration. Myelinization and erratic swimming behavior are symptoms of acute toxicity. This was reported in shrimps reared in biofloc systems using rapidly administered hydrogen peroxide to overcome low oxygen levels due to the absence of aeration (Bögner et al 2021), and the control of oomycetes on all stages and life stages of fish (Dinesh et al 2022).

The concentration and time of exposure are major determinants to determine the degree of toxicity of disinfectant. The recommendation of disinfectants for use in hatchery water and other facilities would be centered on concentration and exposure time.

The pH and dissolved oxygen of water tanks were significantly lower in *C. gariepinus* groups exposed to 21mg/L of chlorine and 21mg/L of hydrogen peroxide in comparison to the control groups. The fishes can become stressed in water with a pH ranging from 4.0 to 6.5 and 9.0 to 11.0 (Ekubo and Abowei 2011). The pH observed in this study was lower than 6.5 leading low survival rate detected in *C. gariepinus* groups exposed to 21mg/L of chlorine and 21mg/L of hydrogen peroxide, respectively.

Dissolved oxygen affects the growth, survival, distribution, behavior, and physiology of fish (Solis 1988). Oxygen depletion in water leads to poor feeding of fish, starvation, reduced growth, and fish mortality, either directly or indirectly (Bhatnagar and Garg 2000). According to Banerjea (1967), dissolved oxygen for average fish production should be above 5 parts per million, and dissolved oxygen 1–3 parts per million has a sublethal effect on survival, growth and feed utilization (Bhatnagar et al 2004). Low dissolved oxygen observed in *C. gariepinus* groups exposed to 21mg/L of chlorine and 21mg/L of hydrogen peroxide may be as a result of chemical degradation and toxic effect of the chemicals used. The resultant effect of lower pH and dissolved oxygen in C. gariepinus groups exposed to higher dose of chlorine and hydrogen peroxide led to lower survival rate. In contrast, the pH and dissolved oxygen of water tanks in C. gariepinus groups exposed to sodium chloride were similar to the control group. However, the normal level of pH and dissolved oxygen in *C. gariepinus* groups exposed to higher dose of sodium chloride led to higher survival rate.

The packed cell volume, RBC, Hb, and heterophil counts were significantly lower in *C. gariepinus* groups exposed to chlorine in an increasing order and in a dose-dependent manner, Specifically, *C. gariepinus* exposed to 21mg/L of chlorine showed lower PCV values, this may infer that higher dose of chlorine involved in breakdown of components of blood resulting in a decrease in the amount of blood in the body of exposed fish. In addition, the loss of blood cells was probably caused by cell destruction and failure of blood production to recompense for the blood loss. The severe anaemia observed in higher the group exposed to higher dose of chlorine may be due to renal damage thereby preventing erythropoiesis (Adeyemi et al 2014, Achilike and Wusu 2019, Anifowose et al 2024b). Moreover, heterophils also showed decreased in values which was an indication in response to challenge. Heterophils are important for intrinsic defense mechanism of the host against pathogens and toxins. Previous studies reported heterophils are agents of innate immune defense and also involved in maintenance of homeostasis. Heterophils are first leukocytes engaged in inflammatory sites and coordination of subsequent adaptive mechanisms. The significantly lower number of neutrophils in the blood in the group exposed to higher dose of chlorine implied a probable collapse of the host defense system. The lower value of heterophils in the exposed group to higher dose of chlorine indicated possibly compromised which indicate that some of the defense forces (neutrophils) are possibly compromised in the fight to protect the host fish from attack (Flerova et al 2013, Buchmann 2022). Monocytes were not significantly different in all the groups exposed to chlorine compared to the control groups. This may infer a mild breakdown of monocytic cells in the exposed fish to chlorine.

In contrast, WBC counts and lymphocytes were significantly higher in *C. gariepinus* groups exposed to chlorine in an increasing order and in a dose-dependent manner. Explicitly, *C. gariepinus* exposed to 21mg/L of chlorine showed higher WBC and lymphocytes. This may indicate an increase in phagocytic cells and defense activity against the toxins produced by chlorine. A higher white blood cell counts indicate a response to stress, implies an intrinsic immune activity aimed at fighting the toxins, and a probable surviving strategy for the exposed fish (Dauda 2018). Moreover, in *C. gariepinus* groups exposed to hydrogen peroxide, a similar pattern of lower packed cell volume, RBC, Hb, and heterophil counts was observed in an increasing order and in a dose-dependent manner. In addition, WBC counts and lymphocytes were significantly

higher in *C. gariepinus* groups exposed to hydrogen peroxide in an increasing order and in a dose-dependent manner. Furthermore, in *C. gariepinus* groups exposed to sodium chloride, a similar pattern of moderately lower packed cell volume, RBC, Hb, and heterophil counts were observed in an increasing order and in a dose-dependent manner. In addition, WBC counts and lymphocytes were moderately higher in *C. gariepinus* groups exposed to sodium chloride in an increasing order and in a dose-dependent manner. The haematological profile of *C. gariepinus* groups exposed to chlorine and hydrogen peroxide was parallel to the fish exposed to sodium chloride. However, this showed that chlorine, hydrogen peroxide, and sodium chloride, exhibited a comparable toxicological effect on exposed *C. gariepinus* groups.

The haematology results observed in this study showed that lowest concentration of the three selected disinfectants exposed to *C. gariepinus*caused a slight increase in white blood cell counts. In contrast sodium chloride showed lower significant white blood cell counts. The highest concentration of the hydrogen peroxide showed a significant high increase in white blood cell counts compared to chlorine and sodium chloride. This is an indication of the high toxicity of the disinfectant on *C. gariepinus*. An increase in antibody production as a result of exposure of fish to pathogens or toxicants is related to an increase in white blood cell counts. This increase is due to release from spleen to counter the diseases or toxicants. In addition, intrusion with the immune system is caused by antibodies production due to chemical compounds in insecticides (Chen and Luo 2023).

The groups of *C. gariepinus* exposed to highest concentration of the disinfectants showed decreased level of red blood cell count. This implies haemolysis and damage to red blood cell membrane. This subsequently led to fast replacement of dead red blood cells due to anaemia to avoid cell shrinkage as a result of osmotic alterations of blood (Ismail 2022). Moreover, significant increase in RBC, WBC, HGB, and HCT may be observed in fish after occurrence of hypoxia (Dagoudo et al 2021). Red blood cell is important in oxygen transport to cells and elevated RBC counts ensure that cells continue to receive adequate oxygen for metabolism. Previous studies showed that extreme hypoxia can cause reduced activity and this might be a reflection of the differences in blood respiratory functions of different species (Pei et al 2021, Obirikorang et al 2025).

The results of this study showed a relationship between disinfectant exposure and the induction of micronucleus (MN) in the red blood cells of fish. Occurrence of micronuclei was reported in different fish organs. Micronuclei were associated with environmental factors as well as the rate of cell proliferation (Altwaijry et al 2023). The effect acute concentrations of all three disinfectants on haematology in this study may be related to induction of varying degrees of nuclear abnormalities (NA). Exposure of fish to toxicants and disinfectants in fish can cause nuclear abnormalities as a result of response to genotoxic agents (Akeredolu et al 2023).

Moreover, high level of bioaccumulation of disinfectants in fish available for consumption has raised a public health concern. This bioaccumulation is mostly depended on application of disinfectants in fresh water bodies (Rose 2023) and their use as a source of domestic water supply (Yeşilbudak 2024). Meanwhile, previous studies showed that human exposures to disinfectant are associated with reproductive and developmental defects. The defects may include immunological abnormalities and hematopoietic cancers (Lafta et al 2024). The exposure time of disinfectants was reported to have a relationship with incidence of cancers in humans. The most important one is brain and prostate cancers (Zhang et al 2025). The toxic effects of disinfectant in humans may also be due to their ability to impair anti-oxidative defense by altering redox equilibria (Kunst et al 2023). This might lead to chromosomal aberrations expressed as micronuclei observed in the erythrocytes of the fishes. This might be observed at acute and sub-cellular levels. The findings of this study showed that sodium chloride produced less mutagenic threat on the fish haematology compared to the other disinfectants investigated. This infer the use of sodium chloride for toxicity control, growth enhancement and parasite treatment in freshwater aquaculture. Though, the effects of sodium chloride may vary among species and concentrations (Tavares-Dias 2022).

## Conclusion

The acute toxic effects and alteration of haematological parameters in *C. gariepinus*, observed for all disinfectants assessed, imply that chemicals commonly used for disinfection remain a serious health concern to aquatic organisms. In view of the toxicity of disinfectants to fish, it is important to consider the quantity or amount of concentration to minimize the risk of intoxication of fish through inhalation into their gills, exposure to their skin, and ingestion while feeding. More than ever, as global populations and food demands rise, increased attention needs to be focused on formulating environmentally friendly, more target-specific, and fast-degrading disinfectants to mitigate the potential negative consequences on fish.

## Declarations

**Competing Interests**

The authors did not report any conflict of interest or financial support

### Availability of Data and Materials
The data that support the findings of this study are available on request from the corresponding author.

### Ethical Statement
Handling of experimental Fish, Ethical clearance, and valid approval were obtained from the Animal Care and Use Research and Ethics Committee (ACUREC) of the University of Ibadan in order to use animals for our studies on disinfectants (NHREC/UIACUREC/05/12/2022A).

### Author Contributions
Motivation/Concept: AOA, ORA; Design: AOA, ORA; Control/Supervision: AOA, ORA; Data Collection and Processing: IJN; Analysis and Interpretation: IJN, ORA, AOA; Literature Review: ORA; Writing the Article: ORA, AOA; Critical Review: AOA

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