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*Full Length Research*

# Tolerance of African catfish *Clarias gariepinus* fingerlings to chemotherapeutic doses of potassium permanganate

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**ABSTRACT:** In this study, the tolerance of *Clarias gariepinus* finger-lings (15.5 g) to chemotherapeutic doses of potassium permanganate was investigated. The experiment was done in 15 plastic tanks (106 each) in hatchery facilities by testing ten doses of potassium permanganate in two experimental batches; 1.0, 3.0, 5.0, 7.0, and 0.0 mg/L in batch one and 0.5, 2.0, 4.0, 6.0, and 0.0 mg/L in batch two, for three duration periods (0, 3 and 6 days) in three replicates. Blood samples were obtained and parameters such haematocrit, haemoglobin concentration, red blood cells (RBCs), and white blood cells (WBCs) were measured. The internal parasites in organ tissue and blood were investigated and the mortality and survival rate were evaluated. Empirical data of the results obtained were subjected to statistical analysis using two-way ANOVA. The results of analysis data showed high variation ($p<0.05$) in the hematological profiles of treated fish under varying levels of potassium permanganate and exposure duration periods (15.24 to 9.74, 32.04 to 18.74, 8582.0 to 13140.0 and 9.11 to 3.71 for Hb, PCV, WBCs and RBCs respectively), which resulted in varying levels of stresses and some biological, biochemical and physiological changes in the fish. The investigation of blood and internal tissues parasites revealed no infectious cases ($p<0.05$). These results show that potassium permanganate can negatively affect the hematology of *Clarias gariepinus*, leading to various stress levels with serious deterioration of fish health and a dramatic increase in mortality rate. It is recommended that 2 to 3 mg/L concentrations of potassium permanganate are suitable for treatment without causing tolerance and at the same time can be used in controlling external infection.

**Keywords**: Chemotherapeutic, *Clarias gariepinus*, fish stress, Potassium permanganate.

## INTRODUCTION

In fish farming practices, many species can tolerate the adverse conditions that result from high density and poor management. African catfish, *Clarias gariepinus* seems to be a suitable species facing such problems. In Sudan, *C. gariepinus* has gained appreciation and plays an important role aquaculture, with high commercial value and acceptance among local consumers (Hagar, 2017). This species grows well under a proper feeding regime with optimum density and an intensive management model. In poor management, the exposure of the fish to stress and disease is expected. Such stress may affect the survival and growth rates of the fish, and these stresses can also affect the production, profitability, and sustainability of the farm. Conventional approaches used to mitigate such stresses and diseases, include the use of disinfectants and enhancement drugs. Blanket treatments are also often used in water as a bath or in feed as additives. For example, the use of potassium permanganate chemotherapeutics for the treatment of parasites in fish has been tested. However, most of the therapeutic dosages used in catfish were based on studies conducted on fish of marketable size with economic values. Only a few studies have been conducted regarding the use of chemotherapeutics in the early life stages of fry and

fingerlings of catfish.

Potassium permanganate ($KMnO_4$) is a chemical oxidizing agent that will react with any organic matter in a pond including algae, bacteria and fish particulate and dissolved organic matter, and organic bottom sediments. Over doses of potassium permanganate may cause stress problems, such as deterioration of fish health and also decrease dissolved oxygen concentrations by killing the algae that produce much of the oxygen in ponds (De Souza and Bonilla-Rodriguez, 2007). For most fish, potassium permanganate can be administered at a concentration of 2 mg/L as a long-term bath. Certain cichlids, are sensitive to potassium permanganate and lower concentrations (1 mg/L) may be safer (De Souza and Bonilla-Rodriguez, 2007). Potassium permanganate can also be used as a short-term bath at concentrations of 10 mg/L for 30 minutes, and in surface disinfectants and external parasite removal at concentrations from 10 mg/L (30 to 60 minutes of contact time) to 500 mg/L (30 seconds of contact time) in fish hatcheries or closed systems (De Souza and Bonilla-Rodriguez, 2007). The effect of potassium permanganate addition on haematological parameters indicated toxicologically and pathophysiologically by environmental monitoring and assessment of fish health conditions (Shah and Altindag, 2004). Nussey et al. (1995) found that hematologic analysis can enhance fish farming by facilitating the early detection of situations of stress and diseases that could affect production performance. In a study by Gabriel et al. (2001) on the effect of a sub-lethal concentration of 13 mg/L of potassium permanganate on the blood composition of *C. gariepinus* exposure under intensive condition resulted in blood deterioration and caused anemia. Kori-Siakpere et al. (2009) also recorded haematological changes in *C. gariepinus* following exposure to sublethal concentrations of potassium permanganate. Their observations included abnormal values of haemoglobin, red blood cell (RBC) counts, white blood cell (WBC) counts and the calculated indices of mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) after long periods of exposure. Annune and Ahuma (1998) also observed haematological changes in the catfish *C. gariepinus* following 28 day of exposure to potassium permanganate. The results also included decreased values of haemoglobin, haematocrit and erythrocyte counts. Therefore, in the present study, an attempt has been made to investigate the effect of potassium permanganate as a chemotherapeutic material in cultures of African catfish *C. gariepinus* fingerlings and the tolerance and response to it.

## MATERIALS AND METHODS

### Experimental layout

The experimental unit consisted of 15 plastic tanks (106 L each). A proper water supply and drainage were fixed in each tank. Five treatments, with three replicates of potassium permanganate in two batches, were tested. The concentrations of potassium permanganate were as follows: 1.0, 3.0, 5.0, 7.0, and 0.0mg/L of $KMnO_4$ in batch one and 0.5, 2.0, 4.0, 6.0, and 0.0mg/L of $KMnO_4$ in batch two for three duration periods (0, 3 and 6 days).

### Experimental fish

Ten African catfish fingerlings (15.5 to 23.6 g) were stocked in each tanks after being acclimatized in a well-conditioned environment. Experimental fish were fed two times a day with commercial feed at (10%) of their body weights.

### Preparation of stock solution

For the stock solution preparation, 1 g of potassium permanganate was added to 100 mg of distilled water (100% concentrate). From this stock solution, various concentrations were prepared by dilution to be used in the investigations.

### Water quality test

The treated water was exchanged every 2 days to maintain the toxicant strength and the level of water quality during the experiment. The water quality parameters (pH, temperature, and total ammonia) of the experimental tanks were measured at every sampling time using different digital measurement methods according to APHA (1998) procedures.

### Blood samples

From selected fingerlings, samples were taken from the end of the body at the caudal peduncle and drops of blood were added to blood collection tubes (EDTA (K3) blood collection tubes). These samples were used for the measurement of some blood parameters such as haematocrit, haemoglobin concentration, RBC count and total WBC count during the sampling period. For testing blood parameters, the most modern complete blood count analyzers were used and provides extended differential counts.

### Blood parasites

From the same selected sample, a blood smear or film was made by dropping two drops of blood onto a glass slide. A drop of blood was put near one end of a dry clean slide and spread with the edge of another smooth clean slide. The film was left to dry in air for 5 minutes. Staining was performed with alcohol (ethanol) and then dilution of the

**Table 1.** Effects of potassium permanganate (0.0; 1.0; 3.0; 5.0 and 7.0 mg/l) on some hematological parameters in *C. gariepinus* finger-lings.

| Blood parameter | Concentration (Mg/l) | Exposure duration period (day) | | |
|---|---|---|---|---|
| | | 0 | 3 | 6 |
| Hb (g/dL) | (0.0) | 15.35$^{a}$±(0.59) | 15.24$^{a}$±(0.65) | 14.92$^{a}$±(1.19) |
| | (1.0) | 15.10$^{a}$±(0.20) | 12.72$^{b}$±(0.63) | 11.26$^{b}$±(0.27) |
| | (3.0) | 15.25$^{a}$±(0.99) | 12.67$^{b}$±(1.24) | 9.13$^{c}$±(0.63) |
| | (5.0) | 15.10$^{a}$±(0.59) | 10.99$^{b}$±(0.96) | 9.74$^{bc}$±(0.49) |
| | (7.0) | 15.31$^{a}$±(0.51) | 9.79$^{b}$±(0.87) | - |
| PCV (%) | (0.0) | 31.78$^{a}$±(0.34) | 32.04$^{a}$±(0.32) | 32.68$^{a}$±(1.55) |
| | (1.0) | 32.88$^{a}$±(1.11) | 26.15$^{b}$±(1.26) | 20.88$^{c}$±(1.42) |
| | (3.0) | 31.70$^{a}$±(1.11) | 26.18$^{b}$±(1.72) | 20.26$^{c}$±(1.33) |
| | (5.0) | 31.60$^{a}$±(0.53) | 25.27$^{b}$±(1.45) | 18.74$^{c}$±(0.79) |
| | (7.0) | 31.95$^{a}$±(0.16) | 21.21$^{b}$±(1.26) | - |
| WBC (×10$^{3}$) | (0.0) | 8240.33$^{a}$±(37.45) | 8582.00$^{a}$±(221.48) | 8231.50$^{a}$±(139.30) |
| | (1.0) | 8222.00$^{a}$±(103.52) | 8366.33$^{ab}$±(435.82) | 8767.33$^{c}$±(963.66) |
| | (3.0) | 8257.00$^{a}$±(216.01) | 8723.67$^{b}$±(450.08) | 11687.33$^{c}$±(1678.24) |
| | (5.0) | 8270.67$^{a}$±(60.72) | 8993.33$^{b}$±(1030.87) | 13140.00$^{c}$±(509.12) |
| | (7.0) | 8235.33$^{a}$±(144.02) | 9572.00$^{b}$±(695.31) | - |
| RBC (×10$^{6}$) | (0.0) | 9.25$^{a}$±(0.99) | 9.11$^{a}$±(0.13) | 8.61$^{a}$±(0.68) |
| | (1.0) | 9.60$^{a}$±(1.43) | 5.70$^{b}$±(1.18) | 4.48$^{c}$±(1.37) |
| | (3.0) | 9.21$^{a}$±(1.00) | 5.42$^{b}$±(0.55) | 3.70$^{c}$±(0.51) |
| | (5.0) | 9.95$^{a}$±(0.94) | 6.13$^{b}$±(1.27) | 3.61$^{c}$±(0.86) |
| | (7.0) | 9.91$^{a}$±(0.76) | 4.22$^{b}$±(0.37) | - |

Means with similar superscripts in a row are statistically insignificant different ($p>0.05$); those with different superscripts are statistically significantly different ($p<0.05$). Key: Hb: Haemoglobin concentration. PCV: Pack Cell Volume. WBC: White blood cell. RBC: Read Blood Cell.

stain was performed with distilled water, washing the slide in tap water for 10 minutes. It was then left to dry in air. The fixed film was saved in a slide container for later examinations and investigations of the blood parasites.

### Internal organ parasites

Internal parasites in tissues and organs were investigated. Sample fish were placed on their backs and cut open with scissors on the ventral side. The dissection was carefully done to expose all the internal organs of the fish. Careful examination of the whole internal organ system was made to identify the presence of any parasites. Specific internal organs like the stomach, kidney, and liver were removed. These removed samples were placed on slides and examined under a microscope.

### Statistical analysis

Data were analyzed and the results obtained for the triplicates from two experiments (batches) were combined, subjected to statistical analysis using two-way analysis of variance (ANOVA) to test differences between the various levels of sub-lethal concentrations of $KMnO_4$ and exposure periods. Multiple comparisons of the means were analyzed by the Duncan tests. All analyses were performed using (SPSS). Results were considered significant at the 95% confidence level ($p< 0.05$).

## RESULTS AND DISCUSSION

African catfish *C. gariepinus* treated with varying levels of potassium permanganate may show varying levels of stresses. Tables 1 and 2 show high variations in the hematological profiles of treated fish under varying levels of potassium permanganate. In this study attempts were made to determine the effects of potassium permanganate with the examination of some blood parameters that are normally used to assess and study tolerance and health statuses of fish and can be used to diagnosis diseases in African catfish. The fish were exposed to concentrations from 0.0 to 7.0 mg/L with ten doses of potassium permanganate in order to investigate their tolerance levels

**Table 2.** Effects of potassium permanganate (0.0; 0.5; 2.0; 4.0 and 6.0 m/l) on some hematological parameters in *C. gariepinus* finger-lings.

| Blood parameter | Concentration (Mg/L) | Exposure duration period (day) | | |
|---|---|---|---|---|
| | | 0 | 3 | 6 |
| HB (g/dL) | (0.0) | 16.50$^{a}$±(2.46) | 17.14$^{a}$±(1.12) | 16.57$^{a}$±(0.71) |
| | (0.5) | 15.38$^{a}$±(4.69) | 15.04$^{a}$±(0.89) | 11.06$^{b}$±(0.95) |
| | (2.0) | 18.13$^{a}$±(1.09) | 14.57$^{b}$±(0.88) | 10.60$^{c}$±(1.41) |
| | (4.0) | 16.81$^{a}$±(1.33) | 14.03$^{b}$±(2.43) | 10.78$^{c}$±(2.00) |
| | (6.0) | 17.77±(0.31) | - | - |
| PVC (%) | (0.0) | 36.48$^{a}$±(1.24) | 36.21$^{a}$±(1.20) | 36.82$^{a}$±(1.04) |
| | (0.5) | 36.92$^{a}$±(1.95) | 31.11$^{b}$±(2.44) | 24.08$^{c}$±(2.01) |
| | (2.0) | 35.97$^{a}$±(0.08) | 30.93$^{b}$±(0.44) | 25.95$^{c}$±(1.83) |
| | (4.0) | 36.37$^{a}$±(1.39) | 31.02$^{b}$±(2.57) | 22.73$^{c}$±(0.54) |
| | (6.0) | 36.52±(1.37) | - | - |
| WBC (×103) | (0.0) | 8674.00$^{a}$±(569.12) | 8993.33$^{a}$±(10.97) | 9777.33$^{a}$±(292.92) |
| | (0.5) | 9187.67$^{b}$±(177.33) | 10219.67$^{b}$±(402.47) | 13032.67$^{a}$±(573.98) |
| | (2.0) | 9077.66$^{c}$±(174.06) | 11431.33$^{b}$±(752.06) | 14400.66$^{a}$±(825.50) |
| | (4.0) | 8899.67$^{b}$±(112.62) | 9552.66$^{b}$±(482.52) | 15352.50$^{a}$±(820.95) |
| | (6.0) | 8931.66±(260.18) | - | - |
| RBC (×106) | (0.0) | 8.22$^{a}$±(1.18) | 9.08$^{a}$±(1.01) | 8.32$^{a}$±(1.23) |
| | (0.5) | 8.29$^{a}$±(0.98) | 5.08$^{b}$±(1.06) | 2.98$^{c}$±(0.75) |
| | (2.0) | 7.09$^{a}$±(0.63) | 4.85$^{b}$±(0.08) | 2.37$^{c}$±(0.33) |
| | (4.0) | 8.89$^{a}$±(0.85) | 4.85$^{b}$±(0.08) | 2.51±(0.27) |
| | (6.0) | 8.23$^{a}$±(0.68) | 4.34$^{b}$±(1.12) | - |

Means with similar superscripts in a row are statistically insignificant different ($p>0.05$); those with different superscripts are statistically significantly different ($p<0.05$). Key: Hb: Haemoglobin concentration. PCV: Pack Cell Volume. WBC: White blood cell. RBC: Read Blood Cell.

and changes in biochemical and haematological parameters of *C. gariepinus* comparing to control conditions. The investigated biochemical blood parameters showed significant ($p<0.05$) differences when compared to untreated fish. The mean values of hematological parameters in *C. gariepinus* fingerlings were informative (Tables 1 and 2). ANOVA indicated that the values of hemoglobin concentration (Hb%), RBC count and packed cell volume (PCV) were significantly decreased (from 15.35, 9.95 and 32.88 to 9.13, 3.61 and 18.74, respectively) at 6 days with further decreases down to the lowest values. Conversely, the WBC count increased (from 8270.67 to 13140.00). Similar results obtained by Kori-Siakpere et al. (2009) revealed changes in hematological parameters of *C. gariepinus* due to stress caused by environmental pollutants, disease, or pathogenic attacks. This was reported by a number of other authors, as well (Onusiriuka and Ufodike, 2000; Ezeri, 2001, Gabriel et al., 2001). Another study by Musa and O'Brien (2001) indicated the reduction in hematological indices of *C. gariepinus* after exposure to sub-lethal concentrations of a common therapeutic malachite green. O'Brien, (2001) also made similar observations when *Oreochromis niloticus* was exposed to sublethal concentrations of formalin.

On the other hand, WBC count were significantly increase with the increased of treatment dose. Many studies found blood parameters to be a sensitive and reliable indicator of environmental stress in fish (Hattingh, 1976; Roche and Boge, 1996). In according with the findings of the present study, a typical response of fish to the effects of acute stress includes a rapid decrease of Hb%, PCV, and RBCs and a dramatic increase in WBCs (Kori-Siakpere et al., 2011). Reductions in Hb%, PCV, and RBCs were observed in the experimental fish exposed to various concentrations of potassium permanganate on the first day, followed by increases at 3 days and 6 days in a regular or gradual way. The initial decrease might be attributed to the great demands and cellular damage that occur in the tissues of the exposed fish and may be a possible cause of protein breakdown (Kori-Siakpere et al., 2011). In general, this study revealed that the total reduction of blood parameters, especially Hb%, PCV, and RBCs, is an indication of anemia caused by exposure of *C. gariepinus* to overly treatment doses of $KMnO_4$ over the study period. According to Blaxhall and Daisley (1973), the

**Table 3.** Mortality and survival percentage of *C. gariepinus* finger-lings exposed to (0.0; 1.0; 3.0; 5.0 and 7.0mg/l) concentrations of potassium permanganate.

| Concentration (mg/L) | Number of fish exposed | Number of mortalities by period | | | Mortality (%) | Survival (%) |
|---|---|---|---|---|---|---|
| | | 0 days | 3 days | 6 days | | |
| (0.0) | 30 | 0 | 0 | 3 | 10 | 90 |
| (1.0) | 30 | 0 | 5 | 2 | 23.33 | 76.67 |
| (3.0) | 30 | 0 | 2 | 1 | 10 | 90 |
| (5.0) | 30 | 0 | 12 | 3 | 50 | 50 |
| (7.0) | 30 | 0 | 28 | 1 | 96.67 | 3.33 |

**Table 4.** Mortality and survival percentage of *C. gariepinus* finger-lings exposed to (0.0; 0.5; 2.0; 4.0 and 6.0mg/l) concentrations of potassium permanganate.

| Concentration (mg/L) | Number of fish exposed | Number of mortalities by period | | | Mortality (%) | Survival (%) |
|---|---|---|---|---|---|---|
| | | 0 days | 3 days | 6 days | | |
| (0.0) | 30 | 0 | 0 | 1 | 3.33 | 96.67 |
| (0.5) | 30 | 0 | 0 | 0 | 0 | 100 |
| (2.0) | 30 | 0 | 0 | 0 | 0 | 100 |
| (4.0) | 30 | 0 | 13 | 0 | 43.3 | 50 |
| (6.0) | 30 | 0 | 30 | 0 | 100 | 0 |

determination of hemoglobin can be a good indicator of anemic conditions in fish. A decrease in hemoglobin concentration after exposure to various concentrations of KMnO4 in the present study confirms that anemic conditions occurred in *C. gariepinus*. Cyriac et al. (1989) considered decreases in hemoglobin concentration as a contribution to hemodilution. The greater reduction at higher concentrations of potassium permanganate may be attributed mainly to the suppression of hemopoietic activity of the kidneys in addition to the increased removal of dysfunctional RBCs from the blood (Kori-Siakpere et al., 2011). Other findings by Buckley et al. (1976) showed that prolonged reduction in hemoglobin content is deleterious to oxygen transport and any blood dyscrasia or degeneration of the erythrocytes could be considered as pathological conditions in fishes exposed to toxicants like potassium permanganate.

Regarding mortality as shown in Tables 3 and 4, the mortality rate was increased with the increase of potassium permanganate concentration, leading to decreases of Hb%, PCV, and RBCs that may in turn cause health deterioration and anemia. The increase of the dose meanwhile led to the increase of WBCs. The suitable doses for treatment and disinfection without stress were 0.5 and 2.0 mg/L, as seen in Table 4. The smaller fingerlings (15.5 g) showed more tolerance (3.33% survival) than the biggest fingerlings (23.6 g) (0% survival). The investigation of internal tissues yielded negative results and no infectious cases were reported.

## Conclusion

This study revealed that *C. gariepinus* finger-lings exposed to various concentrations of potassium permanganate exhibit some biochemical responses. In the hematological profile, the decrease or increase of some parameters led to significant deterioration of the fish culminating in mortality. The results showed that increased of doses of potassium permanganate led to increased mortality and decreased survival rates. The results of the investigation for internal organ parasites may be the effect of increasing and decreasing concentrations of potassium permanganate. As a final observation, the experimental *C. gariepinus* fingerlings have the ability to tolerate various concentrations of potassium permanganate and various environmental condition according to the present study.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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