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Full Length Research Paper

Haematological response of the African catfish: *Clarias gariepinus* (Burchell, 1822) to sublethal concentrations of potassium permanganate

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Potassium permanganate ($KMnO_4$) is a widely used freshwater aquaculture chemotherapeutant for the treatment and prevention of waterborne parasitic and fungal diseases. The goal of this research is to determine the toxicological effects of potassium permanganate on haematological parameters of the wi-dely consumed African catfish, *Clarias gariepinus.* Advanced juveniles *C. gariepinus* were exposed to a sublethal concentrations (0.0, 2.0, 6.0 and 10.0 mg/L) of potassium permanganate for 12, 24, 48, 96 and 192 h adopting the static renewal bioassay technique and subjected to analyses. Blood samples were obtained from the caudal circulation and used for the measurement of haematocrit, haemoglobin con-centration, red and white blood cell counts. Empirical data of the results obtained were subjected to statistical analysis using two-way analysis of variance (ANOVA) to test for level of significance between the various sublethal concentrations of $KMnO_4$ and the exposure periods. Haemoglobin concentrations were significantly ($P< 0.05$) decreased to values between 19.25 and 13.60 mg/dL in all sublethal levels compared to the control value of 19.65 mg/dL at zero time. Haematocrit values were similarly signify-cantly ($P< 0.05$) lowered from the control value of 25.67 to 23.33% in the sublethal levels after 192 h ex-posure. The mean values of the red blood cell count were also significantly lowered from the control value of 1.68 million/mm ^{3} to between 1.64 and 1.15 million/mm^3 in 2.0, 6.0 and 10.0 mg $KMnO_4$/L. Similar trends were observed in the mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) which decreased significantly ($P< 0.05$) with an increase in exposure time, but the level of the mean corpuscular volume was increased. The results suggest that potassium perman-ganate can negatively affect the haematology of fish, causing various disturbances in its health and wellbeing. It is hereby recommended that potassium permanganate widely used in controlling external fungal, bacterial and protozoan infections of fish should not be used indiscriminately.

Key words: Potassium permanganate, haemoglobin, haematocrit, erythrocyte count, haematological indices, *Clarias gariepinus*, Nigeria.

INTRODUCTION

The use of haematological techniques is gaining importa-nce for toxicological research, environmental monitoring and assessment of fish health conditions (Shah and Altin-dag, 2004). Blood parameters are considered patho-phy-siological indicators of the whole body and therefore are important in diagnosing the structural and functional sta-tus of fish exposed to toxicants (Adhikari and Sarkar, 2004; Maheswaran et al., 2008).

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The study of the haematological picture is frequently utilized for the detection of physiopathological changes in different stress conditions (Nussey et al., 1995). Haema-tologic analysis will enhance fish cultivation by facilitating early detection of situations of stress and or diseases that could affect production performance (Rehulka et al., 2004; Tavares-Dias et al., 2005). A number of haemato-logical indices such as haematocrit (Ht), haemoglobin (Hb), total erythrocyte count (TEC) and so on are used to asses the functional status and oxygen carrying capacity of blood stream (Shah and Altindag, 2004).

Kori-Siakpere (1991) experimented on chronic suble-thal effects of copper in a fresh water teleost, *Clarias*

isheriensis; and observed haematological changes resulting from a 90-day exposure to the various sublethal concentrations of copper including decrease in haematocrit and haemoglobin values coupled with a reduction in erythrocyte counts.

Anaemia was also recorded in *C. isheriensis* exposed to sublethal concentrations (0.1, 1.0 and 10 mg/L) of water borne lead; similarly a reduction in plasma electrolytes levels indicative of osmoregulatory impairment in the experimental fish was also recorded (Kori-Siakpere, 1996). Haematological changes in fish such as monocytes and neutrophil counts occur in response to toxicants, irritants or inflammatory conditions and can lead to detrimental effects on fish health (Grizzle, 1977; Ainsworth et al., 1991).

The effect of sublethal concentration of 15 mg/L of malachite green on blood composition of the fish *Clarias gariepinus* exposed under static bioassay also caused anaemia (Musa and Omoregie, 1999).

Annune and Ahuma (1998) recorded haematological changes in *C. gariepinus* following exposure to sublethal concentrations of copper and lead. Their observations included decreased haemoglobin, red blood cell counts, white blood cell count and the calculated indices of mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration(MCHC) after 8 days exposure. Anaemia and haemodilution were implicated in the results.

Kori-Siakpere (1998) also observed haematological changes in the catfish *C. gariepinus* following a 28-day exposure to water soluble fraction of Bonny light crude (petroleum). The results also include decreased values of haemoglobin, haematocrit and erythrocyte counts. Growth of *C. gariepinus* was also altered following the 28-day exposure to water-soluble fraction of Bonny light crude oil indicating that petroleum hydrocarbon could affect the internal organs in addition to the blood of fish (Kori-Siakpere, 2000).

Fish culture is on the increase in Nigeria and the use of potassium permanganate is a management technique in fish production. Varieties of fish diseases, including bacterial disease, are reported to be treated with potassium permanganate. It is claimed that it is useful as a treatment for ectoparasites and fungi at 10 ppm for 10 min or 4 ppm in planted ponds (Tucker and Boyd, 1977).

Therefore in the present study, an attempt has been made to investigate the effect of potassium perman-ganate a commonly used chemotherapeutant in aquaculture management of diseases and parasites; on haemato-logical parameters of the African catfish *C. gariepinus* with particular reference to the concentration of the therapeutant and duration of exposure.

MATERIALS AND METHODS

Apparently healthy live specimens of *C. gariepinus* (mean weight, 165.15 ± 3.45 g; mean length 29.42 ± 6.56 cm) were purchased from Tomab Fish Farms, Obiaruku, Delta State, Nigeria; and were transported to the Animal and Environmental Biology Research Laboratory, Delta State University, Abraka where they were kept in large plastic drums supplied with clean borehole water. Fish were acclimatized to the experimental conditions for two weeks. Mortality during the period of acclimatization was less than 2%.

Stock solution of potassium permanganate ($KMnO_4$) was prepared from 1g standard AnalaR grade granules (BDH Chemicals Ltd., Poole, England) in 1 litre of deionised water to form 100% concentration. From this stock solution, various concentrations used in the investigations were prepared by dilution.

At the end of the acclimatization period, each tank was randomly assigned to one of three treatments (2.0, 6.0 and 10.0 mg/L $KMnO_4$) plus a control. Three tanks were dosed for each testing concentration and control.

The experimental tanks consisted of large plastic containers of 150L capacity, filled to half their capacities and covered with a lid made of fine polyethylene gauze screen of 1mm mesh size, to prevent the fish from jumping out of the containers. Experimental fish were fed daily with Catfish feed (Dizengoff; 4.5 mm; Protein 42%, Fat 13%, Fibre 1.9% and Ash 1.2%) at 3% of their body weights. The fish were not fed 24 h prior to the experimental period, as well as during the experimental period, which lasted 192 h. Natural photoperiod was maintained during the acclimation and experimental period.

The water quality parameters of the experimental tanks were conducted at every sampling time according to APHA (1998) procedures. The water quality parameters measured included pH 6.48 ± 0.32, temperature 28.4 ± 1.2^{o}C, dissolved oxygen 7.36 ± 1.12mgL^{-1}, free carbon dioxide 4.85 ± 0.06 mgL^{-1} and total alkalinity 34.6 ± 1.54 mgL^{-1}.

The test was performed using a semi-static renewal method in which the exposure medium was exchanged every sampling time to maintain toxicant strength and level of dissolved oxygen as well as minimizing the level of ammonia excretion during this experiment.

The sampling was done just before the initial addition of $KMnO_4$ (0 hr = start) and then at 12, 24, 48, 96 and 192 h. Two fish were randomly sampled individually using a small hand net from each experimental tank at each sampling time. The experiments were conducted three times, yielding a total of six fish for each treatment at each sampling time.

Blood from the selected fish was drawn from the caudal vessels with a heparinised disposable plastic syringes and a hypodermic needle. The use of plastic syringe is a necessary precaution with fish blood, because contact with glass results in decreased coagulation time (Smith et al., 1952). The blood samples were then used for the measurement of haematocrit, haemoglobin concentration and red blood cell count and total white blood cell count within 6 h of sampling. All determinations were carried out in duplicates for each sample.

Haemoglobin concentration was measured by the cyanmethaemoglobin method (Larsen and Snieszko, 1961) using a comercially kit (Cromatest Linear Chemicals, Barcelona Spain) . The method is based on the fact that haemoglobin is oxidized to methemoglobin and then to cyanomethemoglobin in a buffered solution containing ferricyanide and cyanide ions. The intensity of co-lour formed is proportional to the amount of haemoglobin present in the sample.

The microhaematocrit method of Snieszko (1960) was used to determine the haematocrit. Blood-filled heparinized microhaematocrit tubes (Hawksley, England) were sealed at one end with plasticine. The tubes were then centrifuged at 12000 g for 5 min using a microhaematocrit centrifuge (Hermle model, Z320; SH 120-1, Shanghai Surgical Instruments, China) and haematocrit values read directly with aid of a haematocrit reader and expressed as a percentage of the blood cells in relation to the whole blood.

The total erythrocyte counts were enumerated in an improved Neubaeur haemocytometer using Yokoyama (1947) diluting fluid. Blood was diluted (1:200) with the diluting fluid in a standard red blood cell pipette and duplicate counts were made for each dilution

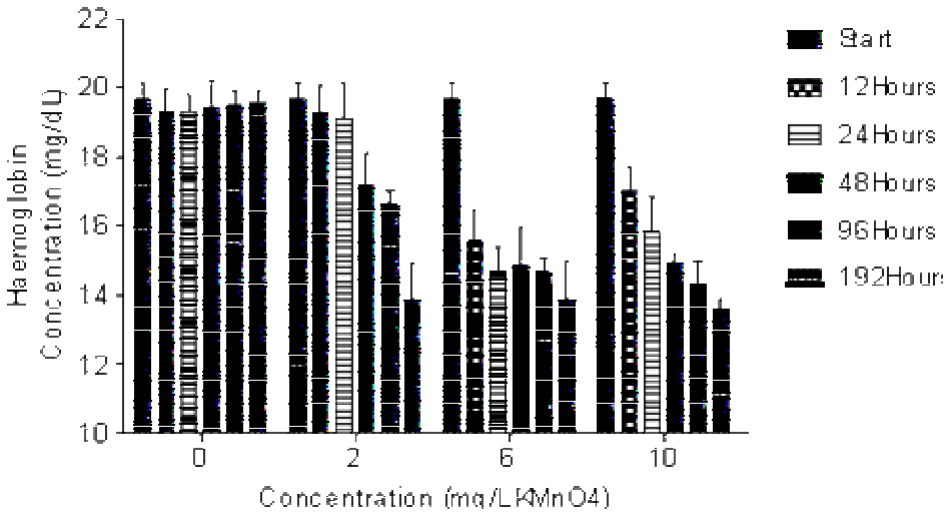

Figure 1. Mean values of haemoglobin in *C. gariepinus* exposed to the various sublethal concentrations of potassium permanganate over a period of 192 h. Each column represents the mean value and vertical bars indicate the standard error of the mean.

Table 1. Percentage variation of haemoglobin of *C. gariepinus* exposed to the various sublethal concentrations of $KMnO_4$ over a period of 192 h.

Concentration	Exposure Period (Hours)					
(mg/L $KMnO_4$)	START	12	24	48	96	192
2	0.00	-0.36	-1.14	-11.77	-4.52	-28.95*
6	0.00	-19.41*	-23.94*	-23.54*	-24.68*	-29.00*
10	0.00	-11.96*	-18.15*	-23.33*	-26.58*	-30.43*

*Indicates significant difference ($P < 0.05$) from the zero time (start) values.

giving the total number of cells per litre. The average of the five counts was reported as the erythrocyte count.

The haematological indices of mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were calculated using the equations given by Anderson and Klontz (1965).

Results obtained for the triplicates from all three experiments were combined, subjected to statistical analysis using two-way analysis of variance (ANOVA) to test differences between the various levels of sublethal concentrations of $KMnO_4$ and the exposure periods. Multiple comparisons of the means were analyzed by the Bonferroni tests. All analyses were performed using the software programme (GraphPads Prism® Software version 5.0, San Diego, CA). Results were considered significant at the 95% confidence level ($P< 0.05$).

RESULTS AND DISCUSSION

The mean values of haemoglobin in *C. gariepinus* exposed to various concentrations of potassium permanganate and at different exposure period is shown in Figure 1; while the percentage variations of haemoglobin from the control values is presented in Table 1. The haemoglobin values ranged from 19.32 to 19.65 g/dL in the control group of the experimental fish. There was a gradual decrease in the mean levels from 19.65 g/dL in the control to 19.25 g/dL in the 2 mg/L $KMnO_4$ exposure group at 12 h; with further decreases down to the lowest value of 13.60 g/dL in the 10 mg/L $KMnO_4$ exposed groups after 192 h. Results of ANOVA showed that there were signify-cant differences in the mean levels values of haemoglo-bin with increase in the concentration levels of the toxi-cant. An aposteriori comparison using Bonferroni tests showed that the mean values of haemoglobin in the treat-ed groups were significantly different from the zero time values especially in the 6 mg/L $KMnO_4$ and 10 mg/L $KMn O_4$ groups, over all the exposure periods. The mean hae-moglobin values in the exposed fish decreased with increase in the exposure period. From a mean level of 19.65 g/dL at the start of the experiment, it decreased by a percentage value of between 0.36 and 30.43. The maximum reduction percentage (-30.43) was recorded in the 10 mg/L $KMnO_4$ treatment for 192 h. The decrease in the mean values of haemoglobin of *C. gariepinus* was a dose and time-dependent.

Changes in haematological parameters of *C. gariepinus* due to stress caused by environmental pollutants, disease or attack by pathogens have been reported by a

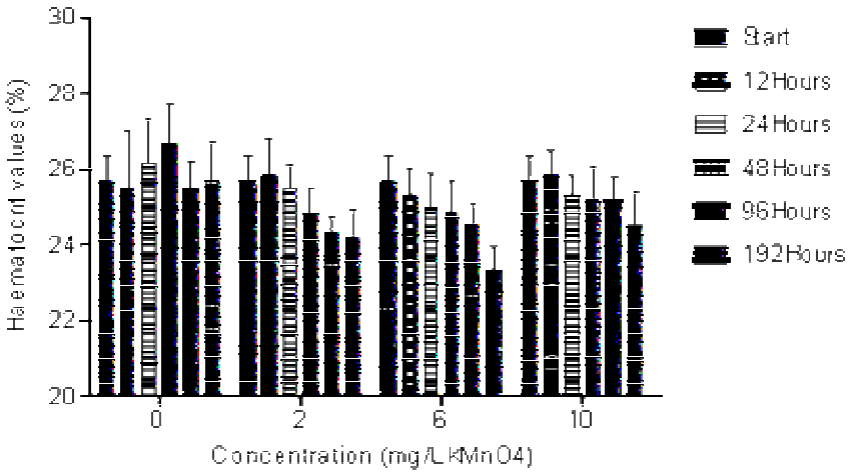

Figure 2. Mean values of haematocrit in *C. gariepinus* exposed to thevarious sublethal concentrations of potassium permanganate over aperiod of 192 hours. Symbols as in Figure 1.

number of authors (Onusiriuka and Ufodike, 2000; Ezeri, 2001, Gabriel et al., 2001). These indices have been employed in effectively monitoring the responses of fish to the stressors and thus its health status under adverse conditions. The results obtained in the present study revealed a significant response in the haematological variables in the potassium permanganate ($KMnO_4$) exposed fish both in respect to concentration and exposure time. The statistically significant ($P < 0.05$) decrease in many values of the haematological parameters studied is not uncommon in fish exposed to sublethal concentrations of toxicants and therapeutic agents. Similar reduction in haematological indices has been reported by Musa and Omoregie (1999) following exposure of *C. gariepinus* to sublethal concentrations often therapeutant: malachite green. Omoregie et al. (1994) also made similar observations when *Oreochromis niloticus* was exposed to sublethal concentrations of formalin. The general reduction of the blood parameters is an indication of anaemia caused by exposure of *C. gariepinus* to the toxicant (therapeutant) $KMnO_4$ over the period of the study.

Haemoglobin is a sophisticated oxygen delivery system that provides the desired amount of oxygen to the tissues under a wide variety of circumstances (Voet and Voet, 19 90) The oxygen transport function of blood is the product of a complex integration of the effects of various physicochemical factors such as temperature and the concentrations of allosteric co-factors, dissolved gases, protons and other ions on the oxygen binding properties of haemoglobin (Weber and Lykkeboe, 1978; Weber, 1982). According to Blaxhall and Daisley (1973) the determination of haemoglobin can be a good indicator of anaemic conditions in fish. A decrease in the haemoglobin concentration after exposure to the various concentrations of $KMnO_4$ in the present study confirms that anaemic conditions occurred in *C. gariepinus*. Cyriac et al. (1989) considered decreases in haemoglobin concentration as a contribution to haemodilution. Haemodilution, being a mechanism that reduces the concentration of the pollutants in the circulatory system (Smit et al. 1979), has been confirmed for aluminium, copper, manganese and zinc (Torres et al. 1986; Wepener, 1990; Nussey 1994; Coetzee, 1996; Barnhoorn, 1996). The decreases in haemoglobin concentration signifies that the fish's ability to provide sufficient oxygen to the tissues is restricted considerably and will result in decrease of physical activity (Grobler 1988; Wepener, 1990; Nussey, 1994). The significant decrease in the haemoglobin concentrations may also be due to either an increase in the rate at which the haemoglobin is destroyed or to a decrease in the rate of haemoglobin synthesis (Reddy and Bashanihideen, 19 89). The progressive reduction in haemoglobin content may also be attributed to depression/exhaustion of haemopoietic potential of the fish (Sawhney and Johal, 20 00). The greater reduction in the higher concentrations of potassium permanganate may be attributed mainly to the suppression of haemopoietic activity of the kidney in addition to the increased removal of dysfunctional red blood cells from blood (Stormer et al., 1996). Buckley et al. (19 76) reported that prolonged reduction in haemoglobin content is deleterious to oxygen transport and any blood dyscrasia and degeneration of the erythrocytes could be ascribed as pathological conditions in fishes exposed to toxicants.

The mean level values of haematocrit in the *C. gariepinus* exposed to various concentration of potassium permanganate under different exposure period are given in Figure 2., while the percentage variations of haematocrit from the control values is presented in Table 2. Haematocrit values of the control group ranged from 25.50 to 26.67%. There was a slight increase in the mean values of haematocrit in the test fish following 12 h exposure

Table 2. Percentage variation of haematocrit of *C. gariepinus* exposed to the various sublethal concentrations of KMnO4 over a period of 192 h.

Concentration	Exposure Period (Hours)					
(mg/L $KMnO_4$)	START	12	24	48	96	192
2	0.00	1.29	-2.56	-6.90	-4.59	-5.84
6	0.00	-0.67	-4.47	-6.90	-3.92	-9.12
10	0.00	1.29	-3.21	-5.62	-1.29	-4.56

Indicates significant difference (P < 0.05) from the zero time (start) values.

Table 3. Percentage variation of haematocrit of *C. gariepinus* exposed to the various sublethal concentrations of KMnO4 over a period of 192 h.

Concentration	Exposure Period (Hours)					
(mg/L $KMnO_4$)	START	12	24	48	96	192
2	0.00	0.00	-5.03	-1.95	-12.26*	-14.10*
6	0.00	-6.75	-7.55*	-9.09*	-13.55*	-26.28*
10	0.00	0.61	-8.18*	-7.14*	-9.03*	-13.46*

*Indicates significant difference (P < 0.05) from the zero time (start) values.

from 25.50% in the control group to 25.83% in the 2.0 mg/L $KMnO_4$ exposed fish, then a slight decrease to 25. 33% in the 6.0 mg/L $KMnO_4$ exposed groups and subsequent slight increase to 25.83% in the 10.0 mg/L KMn O_4 exposed groups. In all the other treatments and expo sure periods, there were slight percentage decreeses ranging between - 2.56 to - 9.12. The maximum reduction percentage (-9.12) was recorded in the 6 mg/L $KMnO_4$ treatment for 192 h. Analysis of variance (ANOVA) failed to detect any significant difference in the mean values of haematocrit in the experimental fish with increase in concentration of the toxicant. Furthermore, a post hoc test (Bonferroni test) confirmed no significant difference in any of the exposed groups. In other words, the control and zero time groups were not significantly different from the mean values of haematocrit of other $KMnO_4$ exposed groups. Overall, the mean values of haematocrit in the exposed fish did not conform to any general pattern but fluctuated slightly with change in the exposure period.

Haematocrit is an important instrument for determining the amount of plasma and corpuscles in the blood (measurement of packed erythrocytes) and used to determine the oxygen carrying capacity of blood (Larsson et al., 1985). It is also defined as the volume occupied by erythrocytes in a given volume of blood and is usually measured as the number of erythrocytes per 100ml of blood. The haematocrit reading is valuable in determining the effect of stressors on the health of fish (Munkittrick and Leatherland, 1983). Significant decreases in the haematocrit values recorded after exposure to $KMnO_4$ are indicative of anaemia and haemodilution possibly due to gill damage or/and impaired osmoregulation (Larsson et al., 1985).

Figure 3 shows the pattern of changes in the mean values of the total erythrocyte count in the fish exposed to various concentrations of potassium permanganate at different exposure time. The percentage variations over the control are listed in Table 3. The result showed control values between 1.54 (x 10^6 mm^{-3}) and 1.68 (x10^6 mm^{-3}). There was a gradual decrease in the mean levels of total erythrocyte count in the test organisms with increase in concentration, which appears dependent on the length of the exposure time. The percentage variation increased with the exposure time in all the treatments. Analysis of variance (ANOVA) result indicated that there was signifycant difference in the mean levels of total erythrocyte count in the exposed fish with increase in the exposure period. However statistical significance (P<0.05) was only recorded after 24 h in the 6 mg/L $KMnO_4$ and 10 mg/L $KMnO_4$ exposed fish. All treatments after 48 h were statistically different from the zero time exposure values. The amount of reduction in total erythrocyte count was al-so higher in the 10 mg/L $KMnO_4$ exposed fish.

Erythrocytes are produced in the haematopoietic tissue, which is situated in the spleen and head kidney (Bond, 1979; Hoffbrand and Pettit, 1980; Smith, 1982; Grey and Meyer, 1988; Kita and Itazawa, 1989; Heath, 1995). It is well known that a reduced quantity and quality of erythrocytes and a decreased haemoglobin level as seen in the present study led to a deteriorated oxygen supply. In addition to transport of oxygen, erythrocytes have other functional tasks in the body; therefore an insufficient quantity and quality of erythrocytes would conesquently have several additional effects on metabolism beyond the simple oxygen supply for tissue metabolism. Prolonged reduction in haemoglobin content has been reported to be deleterious to oxygen transport and any blood dyscrasia and degeneration of the red blood cells

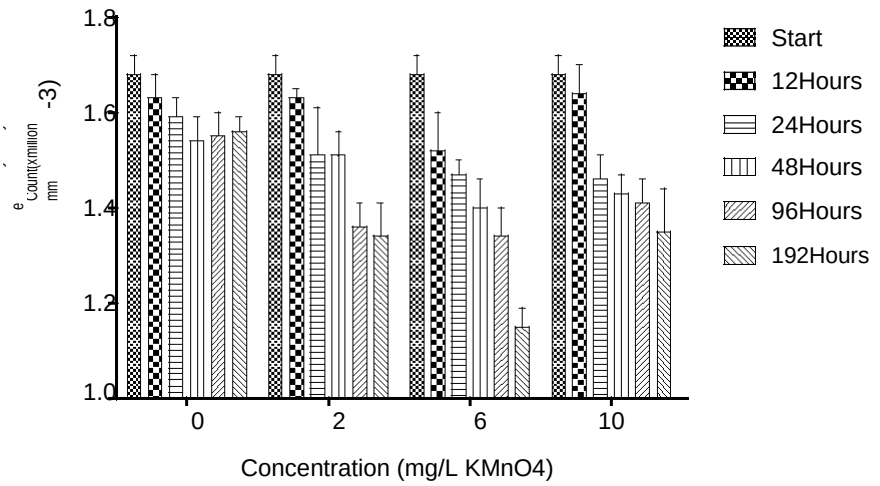

Figure 3. Mean values of total erythrocyte counts of *C. gariepinus* exposed to the various sublethal concentrations of potassium permanganate over a period of 192 h. Symbols as in Figure 1.

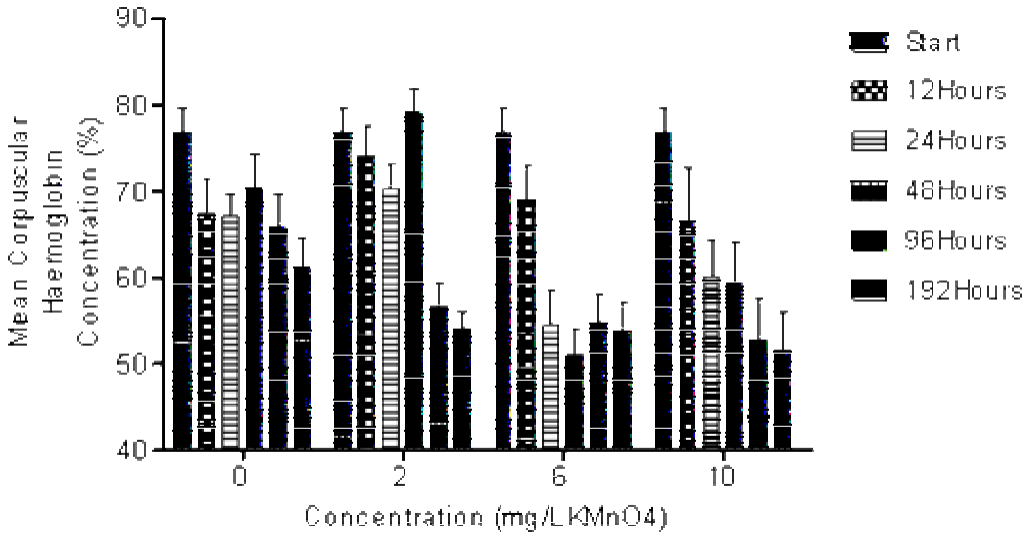

Figure 4. Mean values of mean corpuscular haemoglobin concentration of *C. gariepinus* exposed to the various sublethal concentrations of potassium permanganate over a period of 192 h. Symbols as in Figure.

could be ascribed as pathological conditions in fishes exposed to toxicants (Buckley et al., 1976).

Changes in erythrocyte counts have been reported by Wedemeyer and Yasutake (1977) and Clarke et al. (19 79) to be strong indicators of stress due to presence of toxicants or pollutants in the aquatic environment. Reduction in erythrocytes reported in this study indicated that the experimental fish, *C. gariepinus* became anaemic, which Wedemeyer et al. (1984) attributed to haemodilution resulting from impaired osmoregulation across the gill epithelium. Similar reductions in erythrocytes of Baltic salmon *Salmo salar*, and channel catfish *Ictalurus punctatus*, exposed to malachite green were reported by Glagoleva and Malikova (1968) and Grizzle (1977) respectively; and in Nile tilapia exposed to Gammalin 20 and Actellic 25EC by Omoregie et al. (1990). The reduction in the total erythrocyte count could also be attributed to the destruction of the erythrocytes, thereby limiting their synthesis. Similar trends in erythrocytes in fishes exposed to various toxicants have also been observed by other workers (Mc Leay, 1973; Smit et al., 1979; Koyama and Ozaki, 1984; Srivastava and Narain, 1985; Van der Merwe, 1992).

The mean corpuscular haemoglobin concentration

Table 4. Percentage variation of mean corpuscular haemoglobin concentration of *C. gariepinus* exposed to the various sublethal concentrations of KMnO4 over a period of 192 h.

Concentration (mg/L $KMnO_4$)	Exposure Period (Hours)					
	START	12	24	48	96	192
2	0.00	9.95	4.60	12.40	-3.86*	-11.08*
6	0.00	2.40	-18.84*	-27.29*	-16.62*	-11.67*
10	0.00	-1.62	-10.36*	-15.48*	-19.87*	-15.45*

*Indicates significant difference (P < 0.05) from the zero time (start) values.

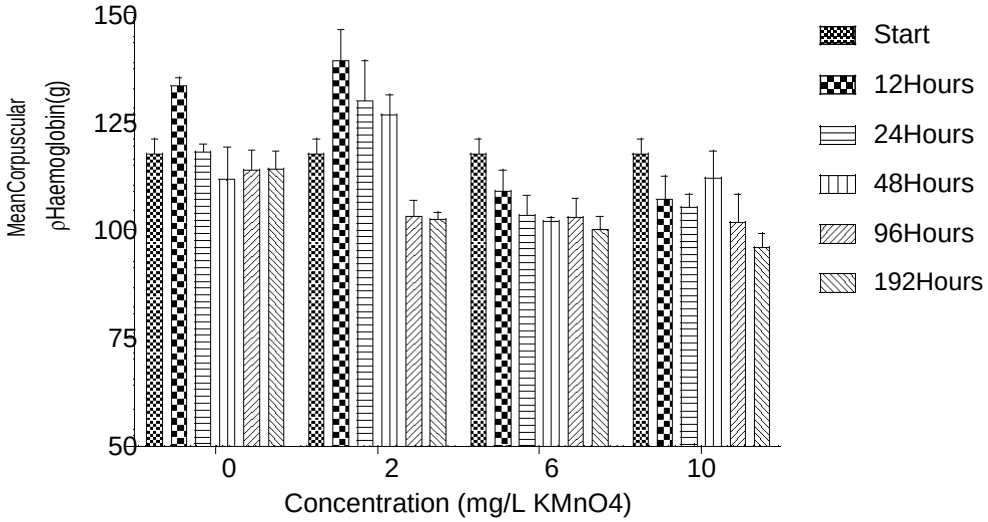

Figure 5. Mean values of the mean corpuscular haemoglobin of C. gariepinus expo-sed to the various sublethal concentrations of potassium permanganate over a period of 192 hours. Symbols as in Figure 1.

values of *C. gariepinus* exposed to various concentrations of $KMnO_4$ under different exposure period is given in Figure 4 with the mean control values ranging from 60. 99 to 76.84%. Similarly, the percentage variations over the control values of mean corpuscular haemoglobin concentration are recorded in Table 4. Though there were slight increases (2.40 – 12.40%) in the mean corpuscular haemoglobin concentration values, majority of the changes experienced were decreased values of the mean corpuscular haemoglobin concentration when compared to control and zero time groups. Analysis of variance (ANOVA) result indicated that there was significant difference in the mean corpuscular haemoglobin concentration with change in exposure concentration and time ($P<0.05$). Bonferroni test showed that the fish exposed to 2 mg/L $KMnO_4$ decreased significantly from the mean levels of the fish in the zero time group following 96 and 192 h exposure. Similarly, there were significant ($P<0.05$) decrease in the fish exposed to 6 mg/L $KMnO_4$ and 10 mg/L $KMnO_4$ from 24 through 192 h.

The values of mean corpuscular haemoglobin in C*. gariepinus* exposed to various concentrations of potassium permanganate under different exposure period are given in Figure 5 result for 10 mg/L for 12 h, while the percentage variations from the control values are presented in Table 5. The mean corpuscular haemoglobin values varied from 114.10 to 129.99 g in the control group of the experimental fish. There was a slight increase in the mean levels from 117.53 g in the zero time groups to 133.09, 129.64 and 126.55 g in the 2 mg/L $KMnO_4$-exposed groups at 12, 24 and 48 h exposure. This was then followed by a decrease of a value of -9.51 and 110. 18 in the 96 and 192 h exposed fish. Generally, there were wide fluctuations in the mean levels of mean corpuscular haemoglobin with increase in the exposure period. This was however not proportional with increase or decrease in time. Analysis of variance (ANOVA) results indicate that there was slight significant difference between the mean levels of mean corpuscular haemoglobin in the exposed fish with change in the exposure time. Analysis of variance (ANOVA) result showed that there was no significant ($P>0.05$) different in the mean levels of MCH in all 2 mg/L treated fish. There was a significant ($P<0.05$) increase in the mean corpuscular haemoglobin

Table 5. Percentage variation of mean corpuscular haemoglobin of *C. gariepinus* exposed to the various sublethal concentrations of KMnO4 over a period of 192 h.

Concentration	Exposure Period (Hours)					
(mg/L $KMnO_4$)	START	12	24	48	96	192
2	0.00	2.38	9.98	13.40	-9.51	-10.18
6	0.00	6.90*	-12.28	-8.55	-9.61	-12.33*
10	0.00	-16.20	-10.75	0.31	-10.63	-15.90*

* Indicates significant difference (P < 0.05) from the zero time (start) values.

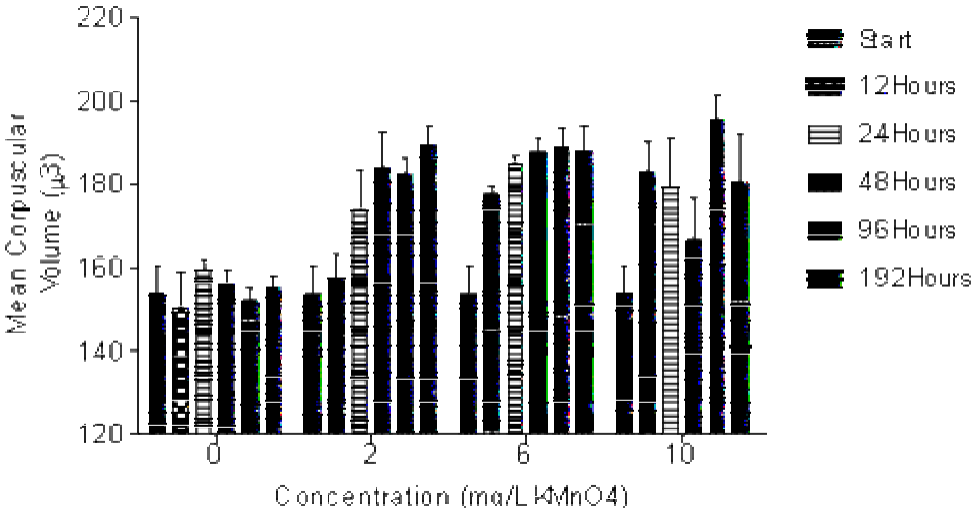

Figure 6. Mean values of the mean corpuscular volume of *C. gariepinus* exposed to the various sublethal concentrations of potassium permanganate over a period of 192 h. Symbols as in Figure 1.

Table 6. Percentage variation of mean corpuscular volume of C. gariepinus exposed to the various sublethal concentrations of KMnO4 over a period of 192 h.

Concentration	Exposure Period (Hours)					
(mg/L $KMnO_4$)	START	12	24	48	96	192
2	0.00	4.7	9.14	17.96*	19.87*	21.98*
6	0.00	18.13*	16.04*	20.38*	23.99*	20.88*
10	0.00	21.63*	12.52*	6.73	28.46*	16.24*

* Indicates significant difference.

of fish exposed to 6 mg/L $KMnO_4$ for 12 h and significant ($P<0.05$) in the fish exposed to 6 mg/L $KMnO_4$ and 10 mg/L $KMnO_4$ for 192 h.

The values of mean corpuscular volume in the test fish exposed to various concentrations of potassium permanganate at different exposure period is given in Figure 6, with the mean control values lying between 150.50 and 159. 29 μ^3. The percentage variation from the control values are presented in Table 6. Analysis of variance (ANOVA) detected significantly difference between the mean values of mean erythrocyte volume and change in concentration of the toxicant in the test organism. However, this change was not proportional to either exposure concentration or period. There was also significant difference ($P<0.05$) in the mean values of mean corpuscular volume in the test fish with increase in exposure period. An posteriori comparison using Bonferroni tests showed that the mean values of mean corpuscular volume in the zero time groups was not significantly different from the 2 mg/L exposed groups at 12 and 24 h exposure and 10 mg/L exposed group at 48 h exposure. In all other treatments and exposure times, there was statistical ($P<0.05$)

difference from the zero time exposure groups. The maximum increase percentage (23.99) was recorded in the mean corpuscular volume of the 6 mg/L exposed groups at 96 h exposure.

The calculated haematological indices, MCHC, MCH, and MCV have a particular importance in the diagnosis of anaemia in most animals (Coles, 1986). The perturbations in these haematological indices (increase MCV, decrease of MCH and MCHC) in the present study may be attributed to a defence against the toxic effect of potassium permanganate through the stimulation of erythropioesis or may be related to the decrease in RBCs, Hb and Hct due to exaggerated disturbances that occurred in both metabolic and haemopoietic activities of fish expo-sed to sublethal concentrations of pollutants (Mousa, 1999). The decrease in MCV coupled with low haemoglo- bin content indicate that the red blood cells have shrunk, either due to hypoxia or microcytic anaemia; microcytosis been due to the decrease in the haematocrit values. The fluctuation in the MCH values clearly indicates that the concentration of haemoglobin in the red blood cells were much lower in the exposed fish than in the control over the exposure period, thus indicating an anaemic condition. The MCHC is a good indicator of red blood cell swelling (Wepener et al., 1992). The MCHC, which is the ratio of blood haemoglobin concentration as opposed to the haematocrit, is not influenced by the blood volume nor by the number of cells in the blood but can be interpreted incorrectly when new cells, with a different haemoglobin concentration, are released into blood circulation (Soivio and Nikinmaa, 1981). The significant decreases in the MCHC values in the exposed fish are thus probably an indication of swelling of the red blood cells and/or a decrease in haemoglobin synthesis.

Haematological parameters related to oxygen transport (Hct and RBCC), defense mechanisms (WBCC) and the calculated indices showed overall differences between control and experimental groups. Therefore, haematological parameters are involved in the response of the African catfish to potassium permanganate under the experimental conditions. The present study thus confirmed that haematological parameters are very sensitive indicators of fish organism response to chemicals in this case potassium permanganate. Meanwhile, the peculiarities of alterations in these parameters depend on the duration of exposure as well as concentrations of chemicals.

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