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Renal and hepatic histopathology of intraperitoneally administered potassium permanganate on *Clarias gariepinus* juveniles

Odo Gregory Ejikeme[1]*, Agwu Ekenma Juliana[1], Eke Felicia[1] and Ikele Chika Bright and Ezea Celestine Osita[2]

[1]Department of Zoology, University of Nigeria, Nsukka, Nigeria.
[2]Federal University of Technology, Owerri, Imo State, Nigeria.

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The biochemistry and histopathology of intraperitoneally administered potassium permanganate was investigated in *Clarias gariepinus*. Acute toxicity of the $KMnO_4$ was determined by intraperitoneally injecting the fish with 0.0, 1.5, 2.0, 4.0 and 6.0 mg/kg. The 96 h lethal concentration (LC_{50}) value obtained from the intraperitoneal injection of the juveniles of *C. gariepinus* with $KMnO_4$ was 2.001 mg/kg $KMnO_4$ at 95% confidence limit. Liver and kidney were excised at the end of each interval of exposure of 0 to 15 days and blood samples were obtained at the end of the exposure period from the caudal ablation and were used for the assay of creatinine, urea and blood urea nitrogen (BUN). The mean creatinine and BUN values differed significantly at $P<0.05$ when compared with the mean values of the control group at the same time. The $KMnO_4$ caused histopathological changes and distortions in the histoarchitecture of the kidney (such as necrotic tubules, cystic spaces, and destruction of renal tubules) and the liver (such as disintegration of hepatic chords, enlargement of the sinusoids, and liver steatosis) of the fish. The potassium permanganate widely used in controlling external fungal, bacterial and protozoan infections of fish should not be indiscriminately used.

Key words: Potassium permanganate, histopathology, biochemical, *Clarias gariepinus*.

INTRODUCTION

Potassium permanganate is a strong oxidant, because of its derivative permanganate ion, MnO^{-2} (Šišperová et al., 2015). It is used as a common biocide at recommended concentrations up to 4 mg/L in various aquaculture setups (kori-Siakpere et al., 2011). Currently, $KMnO_4$ is a U.S Food and Drug Administration (FDA) investigational new animal drugs (INAD) under investigation by Carus Chemical Company Peru. The toxicity margin of $KMnO_4$ is narrow (1 to 3 mg/L) (Plumb, 1999). Toxicity levels have been determined for carp fry as an LC_{50} ranging from 37.5 to 48 mg/L at 26°C and 45 to 37.5 mg/L at 32°C, at 24 and 48 h, respectively (Plumb, 1999; Ezemonye and Ogbomida, 2010). A limited amount of information is available about the toxicity of $KMnO_4$. The

*Corresponding author. Email: gregory.odo@unn.edu.ng.

acute toxicity of $KMnO_4$ to various fish species has been studied (Markings and Bills, 1975; Tucker, 1987).

These include several studies investigating striped bass and the hybrid striped bass (Straus, 2004) and *Clarias gariepinus* Juveniles (Kori-Siakpere, 2008, 2009). Potassium permanganate is poisonous when taken into the blood (Martin, 2003). It consists of dark, odorless, purple crystals with a metallic luster which are soluble in water giving pink to deep purple solutions depending on concentrations (Abalaka, 2013). It is a water disinfectant (Wells, Ponds) and is used as a gastric lavage in alkaloidae poisoning, but promotes rusting. It is an effective algicide (0.01%) and virucide (1%), but concentrations > 1:10,000 tend to irritate tissues (Khan, 2005). Hence, it may contaminate water and can be toxic without prodromal signs; the main lesions are hemorrhage and necrosis of crops in birds. Since potassium permanganate is still used chemically, there is need to access the toxicity of the compound (Brander and Bywater, 1992). The impact of contaminants on the aquatic ecosystem can be measured through a variety of parameters, from low levels of biological organization (molecular and biochemical responses) to high organization levels (population and community responses). In this context, the use of biomarkers at cellular and pathological levels is extremely important as a sensitive tool to measure the biological effect during the assessment of environmental quality, since they are more specific, sensitive, reproducible, and easy to determine. There is no pattern or established limit to this compound and it may be used freely and without restriction in aquaculture. According to the Food and Drug Administration (FDA), the inclusion of this compound in a regulatory class still depends on further studies. Several studies on the implementation and effectiveness of potassium permanganate in aquaculture are available in the literature, but information about its toxic potential in organs and tissues when intraperitoneally injected in fish is still scanty. The impact of contaminants on the aquatic ecosystem can be measured through low levels of biological organization (molecular, biochemical and histopathology) to high levels (community and population response) (FAO, 2010). In this context, histopathology and biomarkers are used as sensitive tools to measure the biological effects during the assessment of environmental quality, since they are more specific, sensitive, reproducible and easy to determine. The general objectives of the study were to determine the LC_{50} of intraperitoneally administered potassium permanganate on *C. gariepinus* juveniles, ascertain the histopathological, and biochemical alterations in the liver and kidney in *C. gariepinus* Juveniles intraperitoneally administered with potassium permanganate.

MATERIALS AND METHODS

Procurement of the experimental fish

The juveniles of *C. gariepinus* of average weight 23.4 ± 6.9 g were used in this experiment. The fish were obtained from a commercial fish farmer (Freedom Fisheries) at Enugu. They were transported to the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. Thereafter, they were kept in a well aerated chlorine free tap water at 25°C and acclimated for two weeks in the laboratory before the commencement of the study. The fish was fed with Coppens fish feed containing 55% crude protein and the water quality was maintained daily with a standard test kit.

Collection of the test compound and preparation of stock solution

The test compound, potassium permanganate used in this study was of analytical grade with 99.97% purity and was purchased from Ogige Market, Nsukka, Enugu State, Nigeria. The stock solutions were obtained and serial dilutions were made.

Acute toxicity bioassay and behavioural responses

Acute toxicity bioassay to determine the 96 h LC_{50} values of potassium permanganate was conducted using the standard protocols (APHA, AWWA, WPCF, 2005). The range finding test was carried out prior to determine the concentrations of the test chemical for the definitive test. For the definitive test, 150 fish of mean weight 14.71 ± 13.61 g were randomly distributed into five groups (A to E) and each group was replicated into three with ten fish per replicate and each fish was intraperitoneally injected with 1.5, 0.4002, 4.0, and 6.0 mg/kg. The experiment was set to determine the LC_{50} values of the test chemical. Fish were visually examined daily and considered dead when no sudden swimming in response to gentle touch was observed. Dead fish were removed with plastic forceps and the mortality was recorded at intervals of 24, 48, 72, and 96 h. The $LC_{10\text{-}90}$ values of the test compound ($KMnO_4$) for the fish at 24, 48, 72, and 96 h was determined by probit analysis (Finney, 1971). The behavioural responses of *C. gariepinus* at different concentrations of $KMnO_4$ were observed from 24 to 96 h of the injection.

Sub lethal test

The 96 h LD_{50} value of $KMnO_4$ in the present study was determined to be 2.001 mg/kg. Based on this value, three sub lethal concentrations of 0.1001, 0.2001 and 0.4002 mg/kg corresponding to 1/20, 1/10 and 1/5th of $KMnO_4$, respectively were prepared by serial dilution of the stock solution. A total of thirty acclimatized fish were injected with each of the aforementioned sub lethal concentrations in triplicates of ten fish per replicate. Another set of 30 fish were injected intraperitoneally with 0.20 normal saline and were considered as the normal saline control groups. Control fish specimens were maintained in dechlorinated tap water without intraperitoneal injection with $KMnO_4$ or normal saline. The experiment lasted for 15 days and the liver and kidney of the test fish were excised at intervals of five days and taken for analysis.

Water quality analysis

The water quality parameters (temperatures, pH and dissolved oxygen) of experimental set up with $KMnO_4$ toxicant and control bioassay were monitored by the procedure according to APHA (1998). Before the commencement of the study, the water temperature was 25.5°C, pH 6.5 and dissolved oxygen 5.2 mg/L. The behavioural responses of the fish were noted, such as erratic swimming and gasping of air.

Biochemicals

Blood samples were taken on the fifteenth day of administration of $KMnO_4$. Blood samples were taken in tubes and centrifuged at 3000 rpm for 10 min for serum separation. The serum was stored at -20^o C for further analysis.

Determination of creatinine

Calibration graph for the estimation of creatinine was carried out in a 3 ml reagent mixture containing 1.93 mM metol, 68.8 µM copper and 1 mM acetic acid/sodium acetate buffer of pH 5.4. The reaction was initiated by adding 100 µl of creatinine concentrations. The reaction mixture was allowed to stand for 30 min at room temperature. Absorbance was read at 530 nm (Copper and Bigga, 1961).

Determination of serum urea

This was carried out using Randox kit for serum urea estimation, following the experimental procedures described by the manufacturer. The absorbance was spectrophotometrically read at 546 nm and calculations were made thus;

Urea concentration (mg/dl) = A sample / A standard × standard concentration.

Determination of Blood Urea Nitrogen (BUN)

Urea (1 mg) corresponds to 0.467 mg of urea nitrogen.
Urea nitrogen = Urea × 0.467

Histopathology

The tissue samples liver and kidney were quickly excised from the fish and fixed in bouins fluid. Slices of the organs were quickly prepared for histological examination to show if there were morphological changes in the organs during the treatment. Processing started by parking the tissue in the tissue capsule. The tissues were dehydrated in graded level of ethanol (70 to 100%) in ascending order. Alcohol was changed after soaking the tissue in them for 1 to 2 h. The tissue was cleaned in chloroform and impregnated with paraffin wax and sectioned at 4 to 5 micron thickness. The section was floated on a water bath maintained at 2 to 3°C below the melting point of paraffin wax. They were dried between 15 and 30 min and were stained with haematoxylin and eosin (H&E), dehydrated, cleaned and mounted (DCM) in a mountant, avoiding air bubbles. Photomicrograph was taken using motic camera with ×40 and ×10 objectives.

Statistical analysis

Mean values were analyzed for significant differences ($P < 0.05$) using the analysis of variance (ANOVA). Differences between means were partitioned using the Duncan new multiple range test. Statistical Package for Social Sciences (SPSS) version 16 was used. Probit value was determined from the probit model developed by Finney (1959).

RESULTS

Water quality parameters

Before the commencement of the study, the water temperature was 25.5°C, pH 6.5 and dissolved oxygen 5.2 mg/L.

Lethal concentration (LC_{50}) determination

The mortality of *C. gariepinus* injected with different concentrations of potassium permanganate is shown in Table 1. A dose-dependent increase and a time dependent decrease was observed in the mortality rate, such that as the exposure time increases from 24 to 96 h, the lethal concentration required to kill the fish was reduced. From the results obtained in the acute toxicity bioassay, a total percentage mortality of 20, 60, 90 and 100% were observed at the end of 24, 48, 72 and 96 h, respectively. A probit analysis was carried out with the result shown in Table 1 and LC_{50} value of 2.001 mg/kg was obtained.

Behavioural responses of *C. gariepinus* intraperitoneally administered with $KMnO_4$

The results showed that potassium permanganate affected the behavioural characteristics of *C. gariepinus.* The control specimens were not hyperactive and showed normal swimming patterns and fin movements throughout the exposure period. The normal saline control groups showed some degree of hyperactivity and jerky movements. However, with increasing $KMnO_4$ concentrations and exposure duration time, hyperactivity and jerky movements increased. In contrast, the swimming rate, fin movement and equilibrium status decreased as shown in Table 2.

Changes in the degree of lesions (wounds, cm) in the skin of *C. gariepinus* Juveniles intraperitoneally administered with various concentrations of $KMnO_4$

The effect of the increasing concentration of intraperitoneally administered $KMnO_4$ after 15 days treatment produced a duration dependent significant ($p < 0.05$) increase in the lesion (wound) levels in the skin of *C. gariepinus* Juveniles. Table 3 shows the degree of lesions (wounds) in cm observed during intraperitoneal injection with $KMnO_4$. A one way ANOVA was used to analyze the lesion degrees shown in Table 3 and the differences between means were partitioned using the Duncan multiple range test with SPSS version 16; the mean values observed in Table 4 was thus obtained. Actually, group B administered with 0.1001 mg of $KMnO_4$ had the highest degree of lesion, followed by group C (0.2001 mg $KMnO_4$) and group D (0.4002 mg $KMnO_4$). When compared with the normal saline, control group A was administered with normal saline only. The increase in lesions was time and concentration dependent as shown in Table 3. Lesions did not occur in the control

Table 1. Summary of mortalities in *C. gariepinus* juveniles intraperitoneally administered with $KMnO_4$.

Test concentration	No. of fish per replicate n=30	Log_{10} concentration	Numbers of mortality				Total number of deaths	Total number Survived
			24 h	48 h	72 h	96 h		
	10	-	0	0	0	0		
A Control (0.00)	10		0	0	0	0	0	30
	10		0	0	0	0		
	10		0	1	1	0		
B (1.50 mg/kg)	10	0.18	0	0	2	0	6	24
	10		0	2	0	0		
	10		0	5	3	0		
C (2.0 mg/kg)	10	0.3	0	3	0	0	18	12
	10		3	1	3	0		
	10		3	3	4	0		
D (4.00 mg/kg)	10	0.6	2	2	3	0	27	3
	10		1	4	5	0		
	10		6	3	1	0		
E (6.00 mg/kg)	10	0.78	4	5	1	0	30	0
	10		2	4	4	0		

groups that were not injected with normal saline or $KMnO_4$.

Changes in some biochemical markers in *C. gariepinus* intraperitoneally administered with $KMnO_4$ at the end of the exposure period

The changes in the creatinine levels of intraperitoneally administered $KMnO_4$ of *C. gariepinus* shows that the creatinine levels among the treated groups differed significantly ($P < 0.05$) with group C 0.4002 mg/kg $KMnO_4$ having the highest creatinine level of 0.82 ± 0.04 mg/dl when compared with other groups. However, group B had the lowest creatinine level of 0.61 ± 0.03 mg/dl and the decrease is significantly different at $P < 0.05$. At the same time, the urea levels among the $KMnO_4$ treated groups differed significantly at $P < 0.05$ when compared with the control; it can be deduced that the group B administered with 0.2001 mg/kg $KMnO_4$ had the highest urea levels at the end of the exposure period, with urea level of 41.34 ± 0.40025 mg/dl followed by group A with urea level of 32.19±1.37 and 25.02 ± 2.28 mg/dl. Moreover, the control groups treated with normal saline maintained urea level of 27.44 ± 3.51 mg/dl.

Similarly, the changes in the normal BUN in the $KMnO_4$ treated groups showed that group B treated with 1.5 mg/kg of $KMnO_4$ had the highest BUN value of 19.31 + 0.96, followed by group A (0.1001 mg/kg) with BUN value of 15.06 + 0.64 and group C (0.4002 mg/kg $KMnO_4$) with BUN value of 11.69 + 0.64. The BUN values among the $KMnO_4$ treated groups differed significantly at $P < 0.05$ when compared with the control. The decrease in the creatinine, urea and BUN among the treated groups had concentration independent changes at the end of the exposure period (Table 5).

Histopathological changes in the kidney of *C. gariepinus* Juveniles intraperitoneally administered with $KMnO_4$

Histopathological changes observed in the kidney (Table 6) of *C. gariepinus* intraperitoneally administered with $KMnO_4$ showed that the kidney of the control group (normal) exhibited intact tubules (Plate 1); however, the group administered with 0.2 mg/kg of normal saline showed necrotic tubules, and destruction of tubules (Plate 2) intact and severe degeneration of tubules as well as intact sinusoids and vacuolations, Plate 3 and cyctic spaces (Plate 4) were seen in days 5 and 15; therefore, the kidney in group B administered with 0.1001 mg/kg $KMnO_4$ showed partial destruction of renal tubules (Plate 5) in day 5, whereas atrophy of the proximal and distal convoluted tubules and massive cystic spaces where evident (Plate 6) in day 10. Meanwhile, in day 15, the kidney architecture appeared normal with tubules being intact without any structural damages as shown in Plate 6. However, group C treated with 0.2001 mg/kg of $KMnO_4$ exhibited increase proliferation of polymorphonuclear cells especially in day 10 (Plate 7),

Table 2. Behavioural changes of *Clarias gariepinus* intraperitoneally administered with various concentrations of $KMnO_4$.

Concentration (mg/kg)	Equilibrium status	Fin movement	Hyperactivity	Jerky movement	Swimming rate
Toxicity test					
24 h					
Control	+++	+++	-	-	+++
1.5	++	++	-	-	+++
2.0	++	++	-	+	+
4.0	-	-	++	+++	+
6.0	-	-	+++	+++	-
48 h					
Control	+++	+++	-	-	+++
1.5	++	++	-	+	++
2.0	++	++	-	++	+
4.0	+	+	++	+++	-
6.0	-	-	+++	+++	-
72 h					
Control	+++	+++	-	-	+++
1.5	++	++	+	+	++
2.0	+	++	+	++	++
4.0	-	+	++	++	-
6.0	-	-	+++	+++	-
96 h					
Control	+++	+++	-	-	+++
1.5	++	++	+	+	+
2.0	+	++	++	++	++
4.0	-	-	+++	+++	-
6.0	-	-	+++	+++	-
Sub lethal test					
24 h					
Control	+++	+++	-	-	+++
0.20 (NS)	++	++	-	-	+++
0.1001	++	++	-	-	++
0.2001	+	+	+	++	+
0.4002	+	+	+	+++	+
48 h					
Control	+++	+++	-	-	+++
0.20 (NS)	++	++	-	+	++
0.1001	++	++	-	+	++
0.2001	+	+	+	++	+
0.4002	+	+	++	+++	+
72 h					
Control	+++	+++	-	-	+++
0.20 (NS)	++	++	+	+	++
0.1001	++	++	+	++	++
0.2001	+	+	++	+++	-
0.4002	-	-	+++	+++	-

Table 2. Contd.

96 h					
Control	+++	+++	-	-	+++
0.20 (NS)	++	++	+	+	+
0.1001	++	++	+	++	++
0.2001	-	-	+++	+++	-
0.4002	-	-	+++	+++	-

- = absent, + = present, ++ = mild, +++ = strong, NS: Normal saline.

Table 3. Various degrees of lesions (wounds) in cm observed in the skin of *C. gariepinus* intraperitoneally administered with normal saline and various concentrations of $KMnO_4$.

Group	Day 5	Day 10	Day 15
A	**-**	**-**	**-**
B	0.3, 0.4, 0.3 cm	1.0, 1.3, 0.5 cm	2.5, 1.5, 1.7 cm
C	0, 0, 0 cm	0.9, 1.0, 0.4 cm	2.0, 1.2, 1.5 cm
D	0, 0, 0 cm	0.9, 1.0, 0 cm	1.8, 1.0, 1.2 cm

Table 4. Changes in the degree of lesions (cm) in the skin of *C. gariepinus* intraperitoneally administered with $KMnO_4$.

Experimental groups	Duration of exposures		
	Day 5	Day 10	Day 15
Control (0.2 mg/kg Normal Saline)	0.00 ± 0.00^b	0.00 ± 0.00^a	0.00 ± 0.00^a
Group A (0.1001 mg/kg)	0.33 ± 0.06^a	0.93 ± 0.40^b	1.90 ± 0.53^b
Group B (0.2001 mg/kg)	0.00 ± 0.00^b	0.77 ± 0.32^b	1.57 ± 0.40^b
Group C (0.4002 mg/kg)	0.00 ± 0.00^b	0.63 ± 0.55^b	1.33 ± 0.42^b

Means with the same alphabets along the column are not statistically significant. Means with different alphabets along the column are statistically significant at $P < 0.05$.

Table 5. Changes in some biochemical markers in *C. gariepinus* intraperitoneally administered with $KMnO_4$ at the end of the exposure period (15 days).

Experimental group	Parameter		
	Creatinine (mg/dl)	Urea (mg/dl)	BUN
Control (Normal Saline)	0.76 ± 0.01^c	27.44 ± 3.51^b	12.81 ± 1.64^b
Group A (0.1001mg/kg)	0.68 ± 0.09^b	32.19 ± 1.37^a	15.06 ± 0.64^a
Group B (0.2001mg/kg)	0.61 ± 0.03^b	41.34 ± 0.40025^c	19.31 ± 0.96^c
Group C (0.4002 mg/kg)	0.82 ± 0.04^a	25.02 ± 2.28^b	11.69 ± 1.06^b

Means with the same alphabets along the column are not statistically significant. Means with different alphabets along the column are statistically significant at $P < 0.05$.

whereas at the end of the treatment, the kidney of the *C. gariepinus* administered with 0.2001 mg/kg $KMnO_4$ appeared to have intact kidney architecture especially at renal cortex of the kidney as shown in Plate 8.

Furthermore, group D administered with 0.4002 mg/kg of $KMnO_4$ had no pathological changes in days 5 and 15,

Table 6. Summarized histopathological effects in the kidney of *C. gareipinus* intraperitoneally administered with $KMnO_4$.

Concentration (mg/kg)	Duration	Necrotic tubules	Tubule disintegration /degeneration	Cystic spaces	Proliferation of polymorphonuclear cells	Vacuolation	Hematopoetic tissue
Control	5	0	0	0	0	0	0
Normal saline (0.2)	5	1	3	2	0	0	0
$KMnO_4$ 0.1001	5	0	1	1	0	0	0
0.2001	5	0	0	0	0	0	0
0.4002	5	0	0	0	0	0	0
Control	10	0	0	0	0	0	0
Normal saline (0.2)	10	0	0	1	0	2	0
$KMnO_4$ 0.1001	10	0	1	2	0	0	0
0.2001	10	3	0	0	0	0	0
0.4002	10	0	0	0	0	0	0
Control	15	0	0	0	0	0	0
Normal saline (0.2)	15	0	3	3	0	0	0
$KMnO_4$ 0.1001	15	0	0	0	0	0	0
0.2001	15	0	0	0	0	0	3
0.4002	15	0	0	0	0	0	0

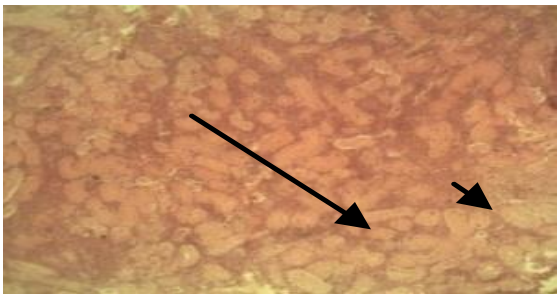

Plate 1. Photomicrograph of control kidney of *C. gariepinus* after 5 days, showing intact tubules (black arrow) (distal and proximal convoluted tubules) and hematopeitic tissue (HT) (black arrow). H&E, magnification x100

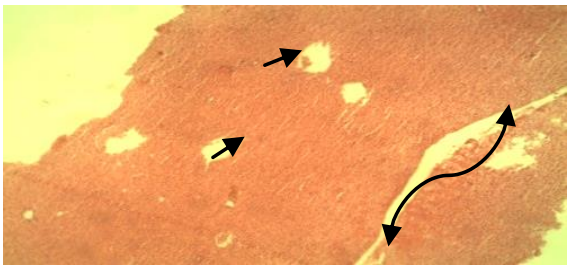

Plate 3. Photomicrograph of *C. gariepinus* liver intraperitoneally administered with 0.2 mg/kg of normal saline after 10 days, showed intact sinusoids (double black arrow head) and vacuolations (black arrow). H&E, magnification x100.

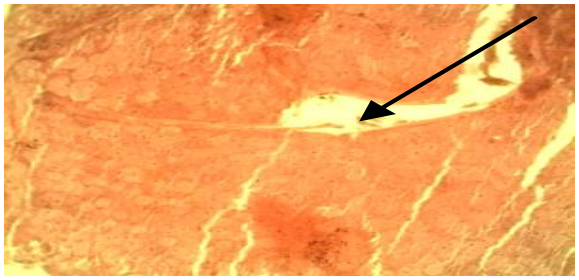

Plate 2. Photomicrograph of *C. gariepinus* kidney intraperitoneally administered with 0.2 mg/kg of normal saline after 5 days, showed necrotic tubules (circles) with destruction of sinusoid tissues (black arrow). H&E, magnification x100.

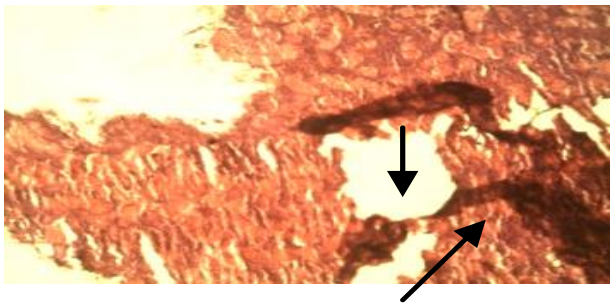

Plate 4. Photomicrograph of *C. gariepinus* kidney intraperitoneally administered with 0.2 mg/kg of normal saline after 15 days, showed severe degeneration of tubules with prominent cystic spaces (black arrows) observed. H&E, magnification x100.

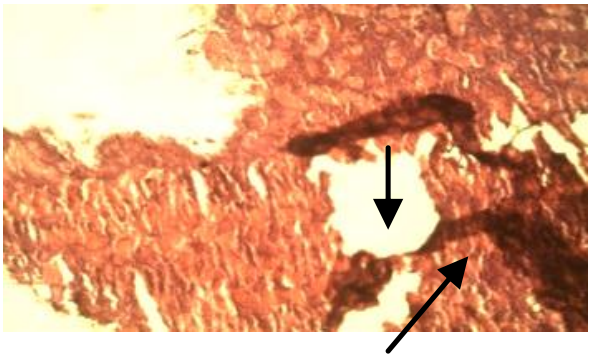

Plate 5. Photomicrograph of *C. gariepinus* kidney intraperitoneally administered with 0.1001 mg/kg of $KMnO_4$ after 5 days, showed partial destruction of renal tubules (circles). However, some of the tubules are intact. H&E, magnification x100.

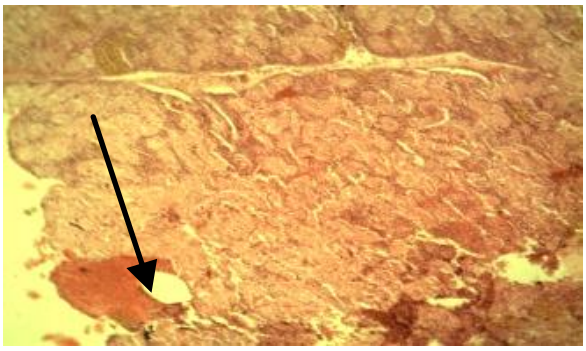

Plate 6. Photomicrograph of *C. gariepinus* kidney intraperitoneally administered with 0.1001 mg/kg of $KMnO_4$ after 10 days, showed atrophied tubules (white star) with small cystic spaces (black arrow). However some tubules are distorted (circles). H&E, magnification x100.

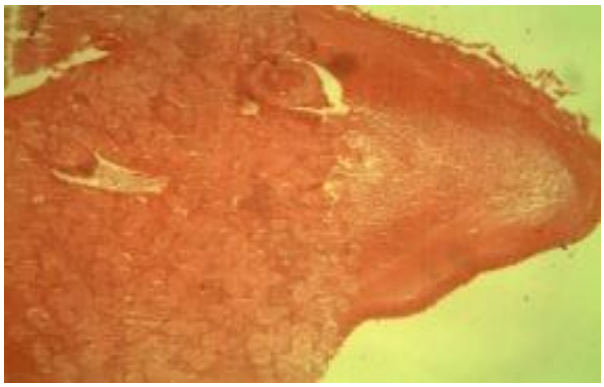

Plate 7. Photomicrograph of *C. gariepinus* trunk kidney intraperitoneally administered with 0.2001 mg/kg of $KMnO_4$ after 10 days, showed proliferation of polymorphonuclear cells (red arrow). However, renal tubules and glomerulus (star) are intact. H&E, magnification x100.

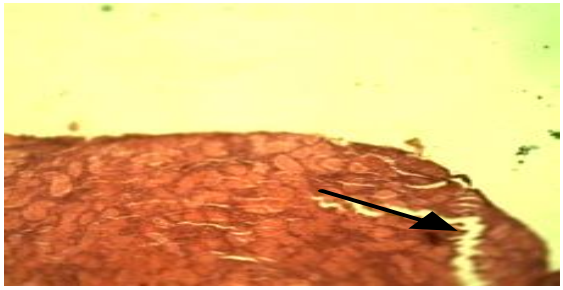

Plate 8. Photomicrograph of *C. gariepinus* typical trunk kidney intraperitoneally administered with 0.2001 mg/kg of $KMnO_4$ after 15 days, showed hematopoetic tissue (white arrow) (HT) and renal tubules intact. H&E, magnification x100.

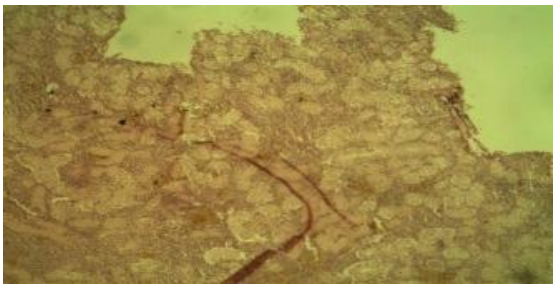

Plate 9. Photomicrograph of *C. gariepinus* kidney intraperitoneally administered with 0.4002 mg/kg of $KMnO_4$ after 10 days, kidney showed no pathological changes. The renal cortex is intact with prominent distal tubules and proximal convoluted tubules. H&E, magnification x100.

the kidney appeared intact, Plate 9.

Histopathological changes in the liver of intraperitoneally administered $KMnO_4$ in *C. gariepinus* Juveniles

The histopathological changes in the *C. gariepinus* Juveniles intraperitoneally administered with $KMnO_4$ showed varied degrees of structural changes in the liver (Table 7). The control liver showed intact parenchymal cells, central vein and sinusoids which usually keeps for the normal structural features seen in the liver (Plate 10); meanwhile, throughout the exposure period especially in the group administered with 0.2 mg/kg of normal saline showed disintegration of hepatic chords Plate 11 which lead to the multiple dilation/or enlargement of the sinusoids (Plate 12) at day 15, whereas, at day 5 no histopathological changes was observed. In addition, there is an onset of liver steatosis due to the visible accumulation of varied sizes of fat droplets (Plate 13) most especially in the group administered with 0.1001 mg/kg $KMnO_4$ in day 10, but in day 15, there was no observable changes in the liver of the fish. Similar fatty

Table 7. Summarized histopathological effects in the liver of *C. gareipinus* intraperitoneally administered with $KMnO_4$.

Concentration (mg/kg)	Duration days	Hepatic cord disintegration	Enlargement of sinusoids	Liver steatosis	Cytoplasmic vacoulation of hepatocytes	Inflammatory cells	Hyperemia
Control	5	0	0	0	0	0	0
Normal saline (0.2)	5	0	0	0	0	0	0
$KMnO_4$ 0.1001	5	2	2	0	0	0	0
0.2001	5	2	1	3	2	0	0
0.4002	5	0	0		0	0	0
Control	10	0	0	0	0	0	0
Normal saline (0.2)	10	3	2	0	0	0	0
$KMnO_4$ 0.1001	10	2	1	3	2	0	0
0.2001	10	0	0	0	0	0	0
0.4002	10	2	1	1	0	0	3
Control	15	0	0	0	0	0	0
Normal saline (0.2)	15	0	0	0	0	0	0
$KMnO_4$ 0.1001	15	0	0	0	0	0	0
0.2001	15	0	0	1	3	3	0
0.4002	15	1	2	1	0	0	0

0: None, 1: mild, 2: moderate, 3: strong.

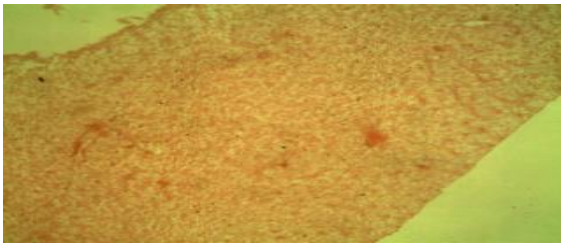

Plate 11. Photomicrograph of *C. gariepinus* liver intraperitoneally administered with 0.2 mg/kg of normal saline after 5 days, showed intact hepatocytes (cirles). H&E, magnification x100.

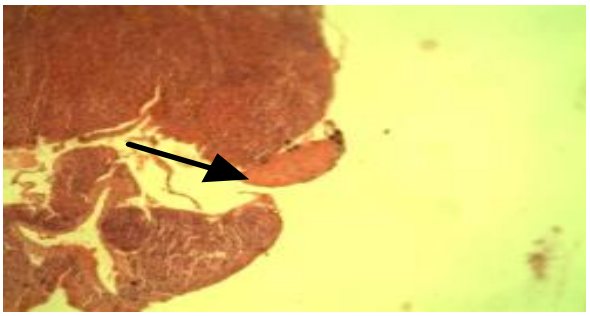

Plate 12. Photomicrograph of *C. gariepinus* liver intraperitoneally administered with 0.2 mg/kg of normal saline after 10 days, showed distorted hepatic chords with dilatation of sinusoids (double head black arrow). However, small bile duct (star) is evident and at the same time, hepatic lobes (red star) can be seen with minor blood congestion (black arrow) in between lobes. H&E, magnification x100.

accumulation (macro and micro vesicular) were also observed in the day 5 of the group administered with 0.2001 mg/kg $KMnO_4$ (Plate 14), while in day 10, the liver appeared to be normal without any visible changes in the architecture as shown in Plate 15. The degree of cytoplasmic vacuolation of hepatocytes and massive inflammatory cells were evident in day 15 in the same group administered with 0.2001 mg/kg $KMnO_4$ (Plate 16). At the highest dose administered to group D (0.4002 mg/kg), the liver tissue showed multiple hyperemias at the end of day 10 exposures (Plate 17) and in day 15, there was a disintegration of hepatic chords (Plate 18).

DISCUSSION

Intraperitoneal administration of $KMnO_4$ to fish inflicts stresses on the mechanisms required to maintain a healthy physiological state. These stresses lead to behavioural changes, and changes in their biochemical and physiological processes. In view of this, there has been increasing interests in examining the physiological, biochemical and histopathological stress responses in aquatic vertebrates to protect aquatic life. The physicochemical parameters of the test were slightly similar during the toxicity test. The percentage mortality observed in the acute study was shown to increase with

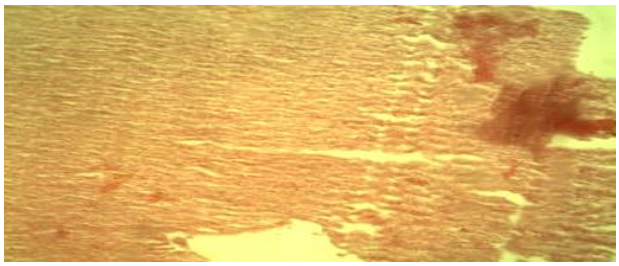

Plate 13. Photomicrograph of *C. gariepinus* liver intraperitoneally administered with 0.1001 mg/kg of $KMnO_4$ after 5 days, showed dilatated sinusoids. H&E, magnification x100.

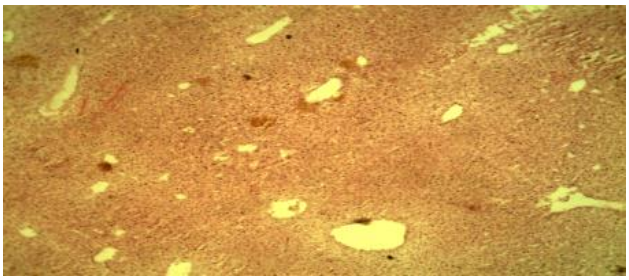

Plate 14. Photomicrograph of *C. gariepinus* liver intraperitoneally administered with 0.1001 mg/kg of $KMnO_4$ after 10 days, liver showed fat accumulation as lipid droplets (micro and macro vesicular fatty change) which usually appears to be consistent with vacuolation of glycogen content.

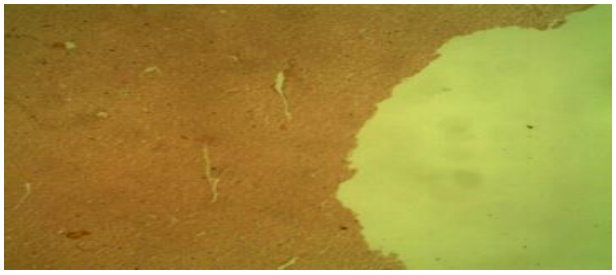

Plate 15. Photomicrograph of *C. gariepinus* liver intraperitoneally administered with 0.2001 mg/kg of $KMnO_4$ after 10 days, showed intact liver architecture with sinusoids and hepatocytes. H&E, magnification x100.

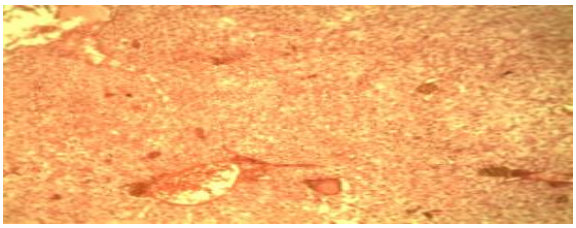

Plate 16. Photomicrograph of *C. gariepinus* liver intraperitoneally administered with 0.2001 mg/kg of $KMnO_4$ after 15 days, tissue showed central vein with infilteration of inflammatory cells (black arrow), traces of bile pigments (red arrow) evident and cytoplasmic vacuolation of heptocytes. H&E, magnification x100.

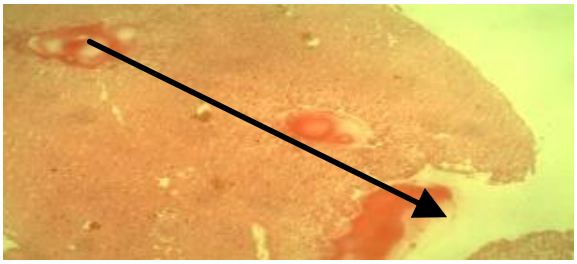

Plate 17. Photomicrograph of *C. gariepinus* liver intraperitoneally administered with 0.4002 mg/kg of $KMnO_4$ after 10 days, showed hyperemia (black arrow). H&E, magnification x100.

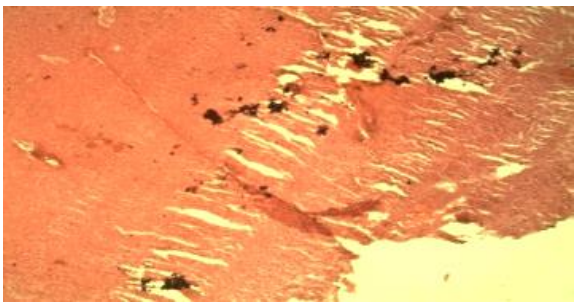

Plate 18. Photomicrograph of *C. gariepinus* liver intraperitoneally administered with 0.4002 mg/kg of $KMnO_4$ after 15 days, showed minor disintegration of hepatic chords (black arrow) evident which led to sinusoid enlargement. H&E, magnification x100. The black stains in the photomicrograph are formalin pigments which can be called artifacts.

increasing doses of the intraperitoneally administered $KMnO_4$. The observation is in consonance with earlier report of Kori-Siakpere (2008). Kori-Siakpere (2008) further reported that the level of any pesticide depends on it bio-accumulation, the different chemistries of the compounds forming the pesticides and the reactions of the organisms receiving the toxicant. The value obtained from the toxicity test in this study agrees with the report of Kori-Siakpere (2008). The water quality parameters may have possibly contributed to the variations in the behavioural pattern and the mortality of the test fish during the study period. During the acute toxicity study, *C. gariepinus* Juveniles were stressed progressively leading to possible observable behavioural changes such as erratic swimming or less of reflex, etc., with time before death. The stressful behavior of respiratory impairment due to the toxic effect of $KMnO_4$ particularly

on the gills may have impaired the respiratory organ. Death could therefore have occurred either by direct poisoning or indirectly by making the medium un-conducive or even by both, whichever is the case, the source of death was potassium permanganate. This demonstrates the observations of Ayoola (2008), that in all toxicants, a threshold is reached above which there is no drastic survival of the animal. Below the threshold, the animal is in a tolerance zone of resistance. The time of toxicity disappearance and mortality were observed from the record of relative mortality time in different concentration of potassium permanganate for 96 h.

The creatinine and urea test has commonly been used to diagnose impaired kidney function and to detect renal damage. However, in the present experiment, there was significant difference in serum creatinine levels in the intraperitoneally administered $KMnO_4$ in *C. gariepinus* Juveniles when compared with the control group. These findings had shown that creatinine may be an accurate biomarker to distinguish dysfunction in kidney tissues. Hence, urea and creatinine are widely used to access renal sufficiency. Higher than normal level of serum urea and creatinine are indications of deficiency in renal function.

Thus, the increase in serum urea concentrations with concomitant increase in serum creatinine concentration in the infected treated animals, suggest that the normal functioning of the kidney has been compromised. Increase in urea level depicts that $KMnO_4$ elicits the elevation. Moreover, the fairly increased levels of serum creatinine in the current study may be induced by glomerular insufficiency, increased muscle tissue catabolism or impairment of carbohydrate metabolism. Histopathological examination of the liver and the kidney of the exposed fish indicated that the kidney and the liver were affected. The liver is the main organ for detoxification (Dutta et al., 1993; Abalaka, 2013). The teleost liver is one of the most sensitive organs with regards to showing alterations in histoarchitecture, biochemistry and physiology following exposure to various types of environmental conditions (Rodrigues and Fanta, 1998). Moreover, in the liver sections of the normal fish, the hepatocytes form a rather cord-like pattern. These cords are arranged around tributaries of the hepatic vein. The liver cells are intact, polygonal in shape with homogenous eosinophilic cytoplasm and centrally located nuclei. Large number of sinusoids appeared intact and slightly separates the cords from another.

The histopathologIcal changes in the liver were more pronounced in days 5, 10 and 15, but the degree of damages varied according to the dosages of $KMnO_4$ administered. The present result showed that $KMnO_4$ induced many histopathological changes in the liver of the catfish *C. gariepinus.* These changes include distorted hepatic chords that led to massive increase in the sinusoids which is usually characterized by widening of hepatic capillaries which may involve the entire lobule or predominantly in the central, periportal or medial areas which can be encountered in different situations. These changes were mainly evident among the groups treated with 0.2 mg/kg normal saline, and 0.1001 mg/kg $KMnO_4$ at the 5th day of exposure. Further, the blood congestion observed in the liver of the test fish in the group treated with 0.2 mg/kg of normal saline in day 10 may be due to the fact that the central vein and sinusoids distended with red blood cells (RBCs) and some areas of hemorrhage are present when RBCs are phagocytized by macrophages. At the same time, these sinusoids clog up thereby blocking the blood from the hepatic artery and interbillary portal vein which have to pass through the sinusoids to get to the central vein. The fatty accumulation in the liver tissues seen as lipid droplets observed among the groups treated with 0.1001 and 0.2001 mg/kg at days 5 and 10, respectively, may be due to abnormal retention of lipids accumulated in the vesicles (a small organelle within a cell consisting of fluid) that displace cytoplasm when the vesicles are large enough to distort the nucleus.

This is keeping for the fact that the liver is the primary organ of lipid metabolism which is most associated with steatosis. This also agrees with Sakr and Gabar (1991) who reported fatty degeneration among large number of cells in *C. gariepinus* exposed to fenvelarte. Desai et al. (1984) reported vacuolation of hepatocytes, fatty degeneration and necrosis in *Tilapia mossanbica* exposed to monocrotophos. There is still paucity of literature on the toxicity of intraperitoneally administered $KMnO_4$ in fishes, especially towards histopathological alterations.

Kidney is the functional unit composed of nephrons. Each nephron is made up of a renal cortex and well developed renal tubules. The kidney of *C. gariepinus* juveniles exposed to $KMnO_4$ showed tubular destruction or fusion of tubules, cystic spaces and necrotic tubules. Renal corpuscles of the kidney were scattered resulting in the disorganization and consequently obstruction to their physiological functions.

These findings agree with the report of Omotoyin et al. (2006). Lesions in the kidney of fish exposed to deltamethrin in the epithelial cells of the renal tubule, pyknotic nuclei in the hematopoeitic tissues, degeneration of glomerulus were observed (Elif, 2006). Whereas, some of the groups especially the group treated with 0.1001 and 0.2001 mg/kg of $KMnO_4$ at days 5 and 10, respectively maintained intact tubules with intact brush border cells. The destruction or degeneration of tubules observed in the present work may be due to autolytic action of lysosomal enzymes released out of these organelles to the ground cytoplasm or due to reabsorption of excreted protein molecules which are generally represented in the glomerular filtrate. More so, it appears that the kidney's tubular cell may possess a transport mechanism similar to that of the hepatocyte (the multi-

specific bile acid transport system) which is responsible for the uptake of the chemical ($KMnO_4$) into the cell.

Conclusion

The results of this study have demonstrated that the use of concentrations considered safe by literature for prophylaxis and treatment of diseases in fish farming (0.1001, 0.2001 and 0.4002 mg/kg $KMnO_4$) may cause considerable changes to the health of exposed fish, thus evidencing the toxic potential of $KMnO_4$ in non target organisms. Such alterations in the histopathology and biochemical markers have pointed out that potassium permanganate can be toxic; therefore, the use of different biomarkers become important as they can reflect more accurately the toxicity of contaminant substances studied and their effects impacting the aquatic ecosystem. It is hereby recommended that $KMnO_4$ widely used in controlling external fungal, bacterial and protozoan infections of fish should not be used indiscriminatingly.

Conflict of Interests

The authors have not declared any conflict of interests.

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