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

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ABSTRACT

Potassium permanganate ($KMnO_4$) is a widely used freshwater aquaculture chemotherapeutant for the treatment and prevention of waterborne parasitic and fungal diseases. The aim of this research was to determine its effects on some biochemical parameters of the widely consumed African catfish, Clarias gariepinus. C. gariepinus were exposed to a sublethal concentrations (0.0, 2.0, 6.0 and 10.0mg/L) of potassium permanganate for 12, 24, 48, 96 and 192 h adopting the static renewal bioassay technique and subjected to blood and plasma analyses. Blood samples were obtained from the caudal circulation and plasma was obtained from blood samples by centrifugation and analyzed spectrophotometrically for plasma biochemistry using Randox kits. Significant dose-dependent increases were recorded in plasma glucose, total plasma protein, plasma cholesterol and plasma triglyceride. The results suggest that potassium permanganate can negatively affect the 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.

Keywords: Potassium permanganate, plasma glucose, total plasma protein, cholesterol, triglycerides, *Clarias gariepinus*, Nigeria.

INTRODUCTION

Biochemical characteristics of blood are among the important indices of the status of internal environment of the fish organism [1 - 2]. For assessment of biochemical indices of fish blood, analysis of blood plasma is preferred to that of blood serum [3]. Changes in the biochemical blood profile mirror changes in metabolism and biochemical processes of the organism, resulting from the effect of pollutants, and they make it possible to study the mechanisms of the effects of these substances[4].

Biochemical and physiological biomarkers are frequently used for detecting or diagnosing sublethal effects in fish exposed to different toxic substances [5]. Sublethal effects are biochemical in origin as the most toxicants exert their effects at basic level of the organism by reacting with enzymes or metabolites and other functional components of the cell. Such effects might lead to irreversible and detrimental disturbances of integrated functions such as behaviour, growth, reproduction and survival [6 - 7].

Many biochemical techniques have been developed in the last few years to allow the detection of the effects of various pollutants on aquatic biota. Some of these are based on clinical cum chemical approaches originally developed to assess human health; while others are derived from basic studies on the mechanism of action of specific toxicants (usually in mammalian systems) [8].

The African Catfish *Clarias gariepinus* is the most suitable species for aquaculture in Africa. *Clarias gariepinus*, which is widely considered to be one of the most important tropical catfish species for aquaculture, has a Pan-African distribution, from Nile to West Africa and from Algeria to South Africa. The African catfish has a high growth rate; it is very resistant to handling stress and is very well appreciated in a wide number of African countries including Nigeria. It is mostly used as an experimental fish since it possesses necessary breathing organs, which enables them to tolerate adverse aquatic and environmental conditions where other cultivate fish species cannot survive [9] because it is hardy and does not easily succumb to death. It inhabits calm waters such as lakes, streams, rivers, swamps and floodplains, some of which are subject to seasonal drying. It is very tolerant to muddy waters and conditions of low dissolved oxygen.

Therefore in the present study, an attempt has been made to investigate the effect of potassium permanganate a commonly used chemotherapeutant in aquaculture management of diseases and parasites; on some biochemical parameters of the African catfish Clarias gariepinus with particular reference to the concentration of the therapeutant and duration of exposure.

MATERIALS AND METHODS

Apparently healthy live specimens of *Clarias gariepinus* (mean weight, 165.15$\pm$3.45g; mean length 29.42$\pm$6.56cm) were purchased from Tomab Fish Farms, Obiaruku, Delta State, Nigeria; and 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 in 1 litre of deionised water to form 100% concentration. From this stock solution, various concentrations used in the investigations were prepared by dilution.

Triplicates of the same experimental concentration design were conducted. For each triplicate, a set of four tanks were stocked with 20 randomly selected fish. At the end of the acclimatization period, each tank was randomly assigned to one of three treatments, plus control. Three tanks were dosed for each testing concentration of potassium permanganate: 2mg/L, 6mg/L, 10mg/L of $KMnO_4$ and one received no $KMnO_4$ (0mg/L; 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; 4.5mm; Protein 42%, Fat 13%, Fibre 1.9% and Ash 1.2%) at 3% of their body weights. The fish were not fed 24hours prior to the experimental period, as well as during the experimental period, which lasted 192 hours. Natural photoperiod was maintained during the acclimation and experimental period.

The water quality parameters of the experimental set up bioassay, with $KMnO_4$ toxicant and control, were conducted at every sampling time according to APHA procedures [10]. The water quality parameters measured included pH 6.48 $\pm$ 0.32, temperature 28.4 $\pm$ 1.2^oC, dissolved oxygen 7.36 $\pm$ 1.12mgL^{-1}, free carbon dioxide 4.85 $\pm$ 0.06 mgL^{-1} and total alkalinity 34.6 $\pm$ 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.

Two fish were randomly caught 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. The sampling was done just before the initial addition of $KMnO_4$ (0hr = start) and then at 12, 24, 48, 96 and 192 hours.

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 [11].

Plasma was obtained by centrifugation and diluted 1:20 with deionised water. The diluted plasma was then stored in a refrigerator at -20^oC and later analysis were conducted for some biochemical parameters: plasma glucose, total plasma protein, cholesterol, triglycerides. All determinations were carried out in duplicates for each sample.

Plasma biochemical parameters of plasma glucose, total plasma protein, cholesterol, and triglycerides were all determined colorimetrically using commercial diagnostic kits (Randox Ltd, UK) following the manufacturer's instruction; using a spectrophotometer (Spectrumlab 21A, Lenjguang Tech, China).

The plasma glucose was measured by using the enzymatic (GOD/PAP) colorimetric method [12] following the enzymatic oxidation of glucose in the presence of glucose oxidase.

The total plasma protein was measured by using the standard Biuret method which is based on the reaction between the peptide bonds of protein and Cu^{2+} (from copper sulphate solution) that produces a blue-violet coloured complex in alkaline solution [13- 14].

The plasma cholesterol was measured by using the enzymatic colorimetric method following the enzymatic hydrolysis and oxidation of cholesterol in the presence of cholesterol esterase and cholesterol oxidase [15].

The plasma triglyceride was measured by using the enzymatic colorimetric method [16] following the enzymatic hydrolysis with lipases. It is worthy of note that 10 mg/dl was subtracted from the triglyceride value obtained to correct for free glycerol.

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

The mean levels of plasma glucose at different concentrations of the exposed fish under various exposure period is shown in Fig.1., while the percentage variations of the plasma glucose in the treated groups are indicated in Table 1.

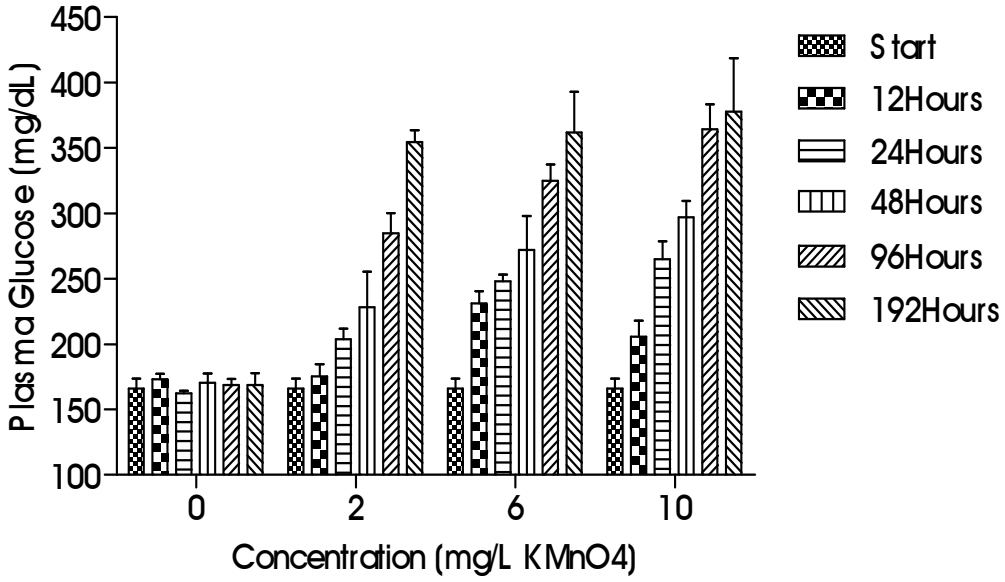

Fig. 1: Mean values of plasma glucose of *C. gariepinus* following exposure to the various sublethal concentrations of potassium permanganate over a period of 192 h. Each column represents the mean value and vertical bars indicate the standard error of the mean.

Table 1: Percentage variation of plasma glucose levels of *C. gariepinus* following exposure to the various concentrations of $KMnO_4$ over a period of 192h.

Concentration (mg/L $KMnO_4$)	Exposure Period (Hours)					
	START	12	24	48	96	192
2	0.00	1.28	25.67*	34.01*	68.90*	110.08*
6	0.00	33.48*	52.88*	59.56*	92.70*	114.32*
10	0.00	18.82*	63.37*	74.41*	116.15*	123.96*

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

The control values of the plasma glucose varied between 162.19mg/dL and 173.10mg/dL. There was significant ($P<0.05$) difference in the mean levels of plasma glucose in the treated fish with change in the concentration of the toxicant and exposure period using analysis of variance (ANOVA) of means. The mean plasma glucose levels in the test fish increased drastically from 165.92mg/dL at the start of the experiment (0hr) to 175.32mg/dL, 231.06mg/dL and 205.67mg/dL after 12 hours exposure period in 2mg/L, 6mg/L and 10mg/L $KMnO_4$ respectively. A post hoc

comparison of means using Bonferroni test showed that the observed increase in plasma glucose in 2mg/L $KMnO_4$ exposed fish at 12hours was not statistically ($P<0.05$) different from the zero time values. Statistically $P<0.05$) significant increases were recorded in all other treatments at all exposure periods. The increase was seemingly dose- and time-dependent with the maximum increase percentage (123.96) being recorded in 10mg/L $KMnO_4$ exposed fish at 192hours.

The mean total plasma protein levels in the control group of *C. gariepinus* exposed to various concentrations of potassium permanganate over a period of 192 hours ranged from 7.58g/dL to 8.43g/dL. The values of the total plasma protein in the treated and control fish are represented in Fig. 2, while changes in terms of percentage of the control values are given in Table 2.

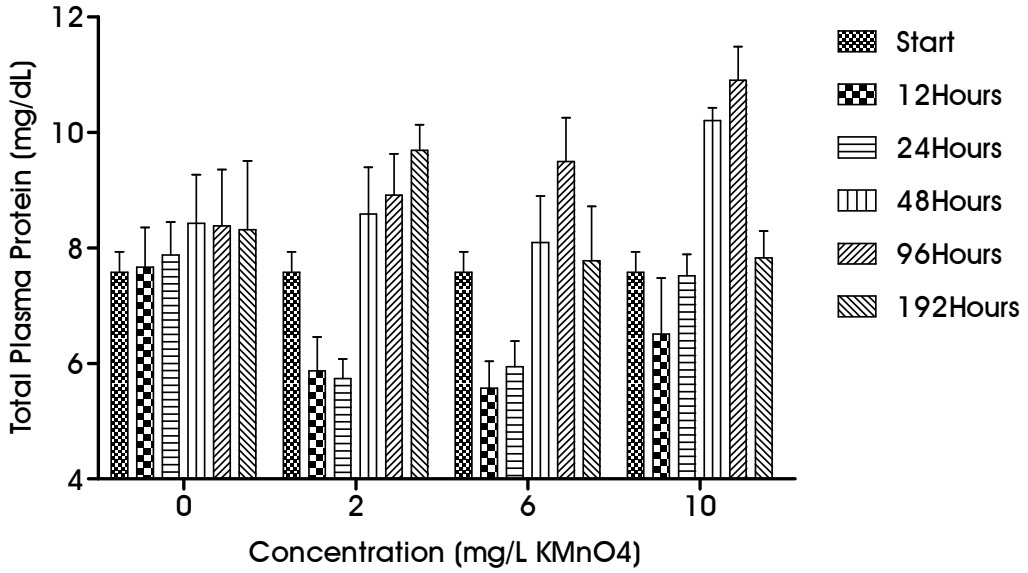

Fig. 2: Mean values of total plasma protein of *C. gariepinus* following exposure to the various sublethal concentrations of potassium permanganate over a period of 192h. Symbols as in Fig. 1.

Table 2: Percentage variation of total plasma protein of *C. gariepinus* following exposure to the various concentrations of $KMnO_4$ over a period of 192h.

Concentration (mg/L $KMnO_4$)	Exposure Period (Hours)					
	START	12	24	48	96	192
2	0.00	-23.47	-27.12	1.90	6.32	16.47
6	0.00	-27.40	-24.62	-3.91	13.23	-6.49
10	0.00	-15.12	-4.57	21.11*	30.04*	-5.89

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

The treated fish showed low values of total plasma protein levels in all treatments at 12 and 24 hours; and the fish exposed to 6mg/L $KMnO_4$ for 48 hours and the fish exposed to 6mg/L $KMnO_4$ and 10mg/L $KMnO_4$ for 192 hours. On the other hand, there were increased total plasma protein levels in all treatments at 96hours; and the fish exposed to 2mg/L $KMnO_4$ and 10mg/L $KMnO_4$ for 48 hours and the fish exposed to 2mg/L $KMnO_4$ for 192 hours. Analysis of variance (ANOVA) result showed that there was no significant difference in the mean levels of total plasma proteins with change in the toxicant concentration. Bonferroni test showed that the mean levels of total plasma protein of the zero time group was only significantly ($P<0.05$) different from the mean levels of the fish exposed to 10mg/L $KMnO_4$ for 48 and 96hours. Thus, no statistical difference was recorded in all the fish in which there were decreased levels.

The mean plasma cholesterol levels in *C. gariepinus* exposed to various concentrations of potassium permanganate at different exposure period and the control values are represented is in Fig.3. The percentage variations with respect to the control values are similarly depicted in Table 3.

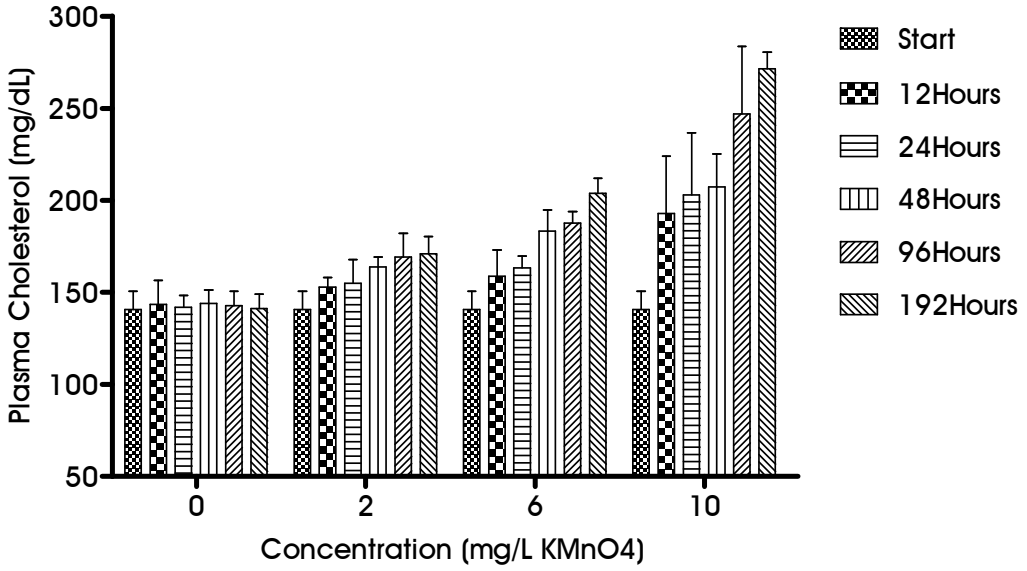

Fig. 3: Mean values of plasma cholesterol of *C. gariepinus* following exposure to the various sublethal concentrations of potassium permanganate over a period of 192h. Symbols as in Fig. 1.

Table 3: Percentage variation of total plasma cholesterol of *C. gariepinus* following exposure to the various concentrations of $KMnO_4$ over a period of 192h.

Concentration (mg/L $KMnO_4$)	Exposure Period (Hours)					
	START	12	24	48	96	192
2	0.00	6.46	9.25	13.71	18.48	21.15
6	0.00	10.70	14.95	27.17	31.46	44.39*
10	0.00	34.37	42.97*	43.86*	72.88*	92.32*

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

The control values of the plasma cholesterol were between 140.82 mg/dL and 144.11 mg/dL. The mean plasma cholesterol levels in the test fish increased slightly from 140.82mg/dL at the start of the experiment (0hr) to 152.77mg/dL, 158.85mg/dL and 192.82mg/dL after 12 hours exposure period in 2mg/L, 6mg/L and 10mg/L $KMnO_4$ respectively. These increases recorded at 12 hours exposure represented percentage values of 6.46, 10.70 and 34.37 with respect to the control values respectively. Generally, analysis of variance result (ANOVA) showed that there was no significant difference ($P<0.05$) in the mean levels of plasma cholesterol in the fish with increase in concentration levels. Nevertheless, plasma cholesterol levels showed significant difference ($P<0.05$) with time. Furthermore, the Bonferroni test for multiple comparison fail to show any significant difference of mean levels of plasma cholesterol in the 2mg/L and 6mg/L exposed fish for all the exposure periods except the 6mg/L $KMnO_4$ exposed fish at 192hours. However, statistical ($P<0.05$) difference was recorded in all 10mg/L $KMnO_4$ treated fish at all exposure times except the 12 hours exposure. The response generally also depicted a dose- and time-dependent trend, with the maximum elevation percentage of 92.32 being recorded in the 10mg/L $KMnO_4$ treated fish.

The mean plasma triglyceride levels in *C. gariepinus* exposed to various concentrations of potassium permanganate at different exposure period is shown in Fig.4, while the percentage variations of plasma triglyceride in treated groups are indicated in Table 4.

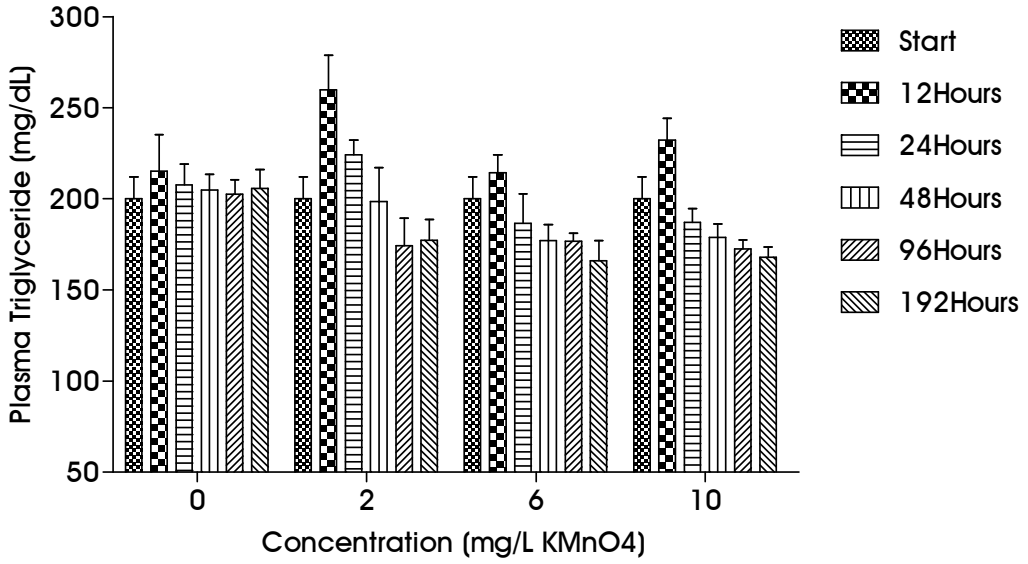

Fig. 4: Mean values of plasma triglyceride of *C. gariepinus* following exposure to the various sublethal concentrations of potassium permanganate over a period of 192h. Symbols as in Fig. 1.

Table 4: Percentage variation of plasma triglyceride of *C. gariepinus* following exposure to the various concentrations of $KMnO_4$ over a period of 192h.

Concentration (mg/L $KMnO_4$)	Exposure Period (Hours)					
	START	12	24	48	96	192
2	0.00	20.74*	7.91	-3.15	-13.93	-13.80
6	0.00	-0.44	-10.15	-13.50	-12.64	-19.30
10	0.00	7.88	-9.84	-12.70	-14.84	-18.28

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

The mean plasma triglyceride levels in the control group varied between 200.20mg/dL and 215.24mg/dL. In *C. gariepinus* exposed to 2mg/L of $KMnO_4$. there was an initial increase in plasma triglyceride levels at 12 and 24 hours; followed by decreases at 48, 96 and 192 hours. The 6mg/L of $KMnO_4$ treated fish at all exposure time exhibited decreased values, while the 10mg/L of $KMnO_4$ exposed fish showed increased plasma triglyceride values following 12 hours exposure and thereafter decreased for all other exposure periods.

Analysis of variance result fail to indicate any significance ($P<0.05$) in the mean levels of plasma triglyceride observed in *C. gariepinus* with increase in the toxicant concentration and exposure time. In addition, a post hoc test using Bonferroni test, only indicated a significant ($P<0.05$) increase in the mean levels of plasma triglyceride in C. gariepinus exposed to 2mg/L $KMnO_4$ following 12hours exposure.

DISCUSSION

Exposure of animals to sublethal levels of toxicants may inflict stresses on the mechanisms required for maintaining a healthy physiological state. These stresses may result in changes in biochemical, physiological or behavioural processes. In view of this, there has been increasing interest in examining the physiological and biochemical stress response in aquatic vertebrates to protect aquatic life. A hand full of biochemical blood parameters are routinely used to assess health status and aid in the diagnosis of diseases in man and animals. These tools in most cases have less frequently been applied in studies dealing with ecotoxicology or related disciplines. In this study, an attempt was made to investigate biochemical blood parameters in *C. gariepinus* following exposure to sublethal concentrations of potassium permanganate in the water. The biochemical parameters investigated showed significant ($P < 0.05$) differences when compared to the control groups.

Plasma glucose is a sensitive reliable indicator of environmental stress in fish [17 – 18]. A typical response of fish to acute stress includes a rapid rise in the glucose concentration: hyperglycaemia has been recorded in the blood of fish after exposure to different stressors [19 – 21]. The stress exerted on the fish causes the release of adrenaline and noradrenaline to activate the secretion of catecholamine [22]. This catecholamine increases the conversion of liver glycogen to glucose to supply the greater energy demand by stress-induced increased metabolism [20]. Such phenomenon may have occurred in experimental fish in the present study, since the plasma glucose increased progressively with increasing concentrations of potassium permanganate and exposure periods. As pointed out by Ghosh [23], these changes in the plasma glucose levels may further be attributable to the changes in the respiration and activity of the fish. Omoregie *et al*. [24] suggested that the progressive accumulation of plasma glucose suggests a stress-induced hyperglycaemia resulting from the incomplete metabolism of blood sugar due to impaired osmoregulation.

One of the important functions of plasma/serum protein is the maintenance of osmotic balance between the circulating blood and tissue fluids [25]. The influence of toxicants on the total protein concentration of fish has also been taken into consideration in evaluating the response to stressors and consequently the increasing demand for energy. Total plasma protein level is a frequently used parameter for metal poisoning in fish. However, data available did not allow to assessment of the direction of the changes, since the same metal may cause both an increase and a decrease in total protein. A reduction in the plasma protein was observed in the experimental fish exposed to the various concentration of potassium permanganate in the first 48 hours; followed by increases at 96 and 192 hours. The initial decrease might be attributed to the great demands and cellular damage that occurred in the tissues of the exposed fish and may be a possible cause of protein breakdown.

Generally, a reduction in plasma/serum protein in fish exposed to different pollutants has been reported earlier [26 – 28]. The higher energy demand of the body to counter stress may trigger an increase in protein catabolism, a process in which both blood and structural protein are converted to energy during toxicant stress thereby reducing the serum protein [27 – 28]. On the other hand, the increase in plasma protein after 96hr exposure could attributed to the hypoxic condition of the under continuous stress. Bouck [29] working with hypoxic stressed rock bass *Ambloplites rupetris*, showed that changes in plasma protein were in part due to entry of proteins from the tissues into the blood. It has been suggested that anaerobic enzymes such as lactate dehydrogenase might become elevated in plasma, increasing plasma protein concentrations, before adaptations occur in the haematological parameters [30]. The increased plasma protein

may also lead to increased osmotic pressure and osmolality of the plasma and resulting from the movement of protein into the cellular compartment [31- 34]. The water shift may be due to an osmotic gradient created by an intracellular lactate accumulation [34].

A dose-dependent increase in plasma concentration of cholesterol was observed in the experimental fish in the present study. This rise in cholesterol is an indication and probably suggests a general increase in lipid mobilization. Hypercholestrolemia observed may be due impairment of liver and inhibition of enzymes which convert cholesterol into bile acid [35]. Reduced lipoprotein lipase activity plays a role in the increment of plasma lipid [36]. Serum cholesterol may also tend to rise with renal plasma, thus causing the concentration to increase markedly. Previous studies with dietary additions of PCB in rats have resulted in increase cholesterol, triglycerides and liver lipids [37-38].

Fish under stress mobilizes triglyceride and protein to fulfill an increased demand for energy to cope with detrimental conditions imposed by the toxicant/xenobiotic and to meet energy required to sustain increased physical activity, bio-transformation and excretion of xenobiotic [39 – 41].

In conclusion, the study revealed that *C. gariepinus* exposed to various sublethal concentrations of potassium permanganate exhibit some biochemical responses. It is therefore recommended that potassium permanganate widely used in controlling external fungal, bacterial and protozoan infections of fish should not be used indiscriminately in aquaculture.

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