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Changes in the Leucocyte and Serum Biochemistry in *Clarias gariepinus* (Burchel) Exposed to Sublethal Lead Choride

* Oluah, Ndubuisi Stanley, Ulasi, A.M Omerebele and Nwani, Christopher Didigwu
Ecotoxicology Research Laboratory, Fisheries & Hydrobiology Reseaech Unit,
Department of Zoology & Environmental Biology, University of Nigeria, Nsukka
email = ndubuisi.oluah@unn.edu.ng; ndubuisioluah@yahoo.com

*To whom all correspondences should be addressed.

Abstract
Clarias gariepinus fingerlings were chronically exposed to sublethal concentrations of lead (0.00; 0.10 and 0.40 mg/L) as lead chloride for twenty eight days in the laboratory. The changes in the leucocytes and serum biochemical parameters (glucose, protein and total cholesterol) of the fish were determined every seven days for 28 days in a renewable static bioassay system. At the end of the study, these parameters were significantly ($p < 0.05$) elevated in the treatment groups when compared with the control. There was pronounced leucocytosis in the lead-exposed fish when compared with the control. The lymphocytes and the basophils were the most dominant agranulocyte and granulocytes, respectively. The magnitude of increase was influenced by both duration of exposure and concentration. The fish exposed to lead were significantly ($p<0.05$) hyperglycaemic and hypercholesteremic. The serum protein concentration was also significantly ($p< 0.05$) increased in the treatment groups when compared with the control. These changes are indications of stress imposed on the fish by lead and could be used as indices of lead poisoning.
Keywords: *Clarias,* lead, hyperglycaemia, cholesterol, protein, leucocyte

1 INTRODUCTION

In recent years heavy metal contamination of the aquatic ecosystem has become a source of concern to not only environmentalists but also to health workers and biologist alike due the public health implications of the increased environmental load of these metals. This problem seemed to be exacerbated by the uncontrolled anthropogenic discharges of heavy metals as industrial wastes, sewage, pesticides and through mining activities.
Lead is one of the most widely used heavy metal that has wide applications in such products as storage batteries (lead accumulator), electric cable sheaths, alloys, pesticides, paints, petrol and rubber products among other uses (Jackson et al, 2005). Naturally, lead occurs in water bodies in trace amounts but due increasing commercial activities, it is increasingly being mobilized into the aquatic environment that the concentration could reach toxic level (Van Vuren, 1999). Lead reaches water bodies either as industrial effluents, runoff from agricultural fields or as ores from mines. In water bodies, lead forms complexes with sediments or organic materials (TNO, 2001) and in the process enters the food chain. The problem posed by lead in the aquatic system is complex due to its non-degradability and interactions with other materials to form complexes that may potentiate their toxic effects. In mammals lead exposure promotes increased liver weight, pycnosis of kupfer cells and inhibited hepatic enzymes (ATSDR, 1999). Once absorbed into the body, the lead affects the fish in a variety of ways. It affects the blood (Allen, 1993; Nussey *et al*, 1995), the structural integrity of vital organs of the fish (Karan *et al*, 1998) and the enzyme systems (Oluah, 1999). Heavy metal contamination also predisposes animals to chromosomal and DNA damage (Palmer and Puls, 1994; Bolognesi *et al*, 1999) as well as neurological, renal and biochemical disorders in fish (Goyer, 1993). In fish, lead induces poor growth performance and reduced nitrogen conversion ratio (Hayat et al, 2007; Naz et al, 2008).

In Nigeria, due to increasing industrialization, pesticide application and other commercial activities, the level of lead ion in Nigerian waters has increased in recent years above WHO permissible levels of 0.2mg/L (WHO, 1996). In Aba River, Mgbemena et al (2011) reported that the lead level ranged from 60.025 -70.16 mg/kg sediment while in Anam River at Otuocha, the concentration ranged from 0.59-7.34 ppm (Igwilo et al., 2006). In Ethiope River, the lead concentration ranges between 0.27-0.72 mg/L (Osakwe and Peretiemo-Clarke, 2013). The level of lead in Kubanni and Delimi Rivers in Northern Nigeria are 6.54 and 0.51-0.95 mg/L, respectively (Uzairu et al., 2008; Sabo et al., 2013). Within the last few years, newspapers in Nigeria reported that more than four thousand eight hundred persons died of lead poisoning in Zamfara State.

The food habit and ecology of *Clarias* species as omnivorous and opportunistic feeders predispose it to lead poisoning as it feeds on both food of animal origin and detritus. Thus, *Clarias* is a good sentinel organism for ecotoxicological study of lead contamination in water bodies. The purpose of the study was therefore to investigate the effect of sublethal lead on the leucocyte and some biochemical parameters in *Clarias gariepinus* with a view to using them as biomarkers of lead toxicity.

2 MATERIALS AND METHODS

2.1 Collection and handling of experimental of fish.

The fish juveniles were bought from Aqua Fish Farms Ltd Awka, Anambra State, Nigeria and transported to our wet laboratory in two 50 L plastic fish transport container in the morning to minimize heat shock due high temperatures that we normally experience in the afternoon. Once in the wet laboratory, the fish was introduced into 450 L plastic container for acclimatization for fourteen days. The water was continually aerated to ensure that the dissolved oxygen level remained above 6 mg/L and the fish was fed on 35% crude protein diet at 3% body weight daily at 8.00 h.

2.2 Experimental design and *in vivo* studies.

Ninety juveniles of *Clarias gariepinus* (mean length of 35.00±2.50 cm and average weight of 150g±5.20g) were used for the study. They were divided into three groups of thirty fish each. Each group was further divided into three replicate experiments containing ten fish per replicate. One group of fish was treated with 0.1 mg/L lead as lead chloride and the second group was exposed to 0.4 mg/L of lead as lead chloride. These test concentrations were chosen after considering the level of lead in some water bodies in Nigeria (Igwilo et al., 2006; Mgbemena et al., 2011; Uzairu et al., 2008; Sabo et al., 2013). The third group was exposed to tap water only and it served as the control. The water in the replicate experiments was changed everyday to maintain the toxicant concentrations. One fish from each replicate experiment was killed every seven days for twenty eight days for the analysis.

2.3 Blood Collection.

The blood of the fish was collected every seven days through both cardiac puncture (El-Sherif et al.,2009). The blood was collected into three different containers. One of the bottles containing fluoride oxalate was use to collect blood used for glucose determination. The other two bottles were without anticoagulant and were used to collect the blood for the biochemical test and thin blood smear.

2.4 White Blood Cell Count (WBCC).

The leucocyte count was done using the Neubauer microscopic counter after diluting the blood with Turk's dilution fluid. The differential white blood cell count was done by preparing a thin blood smear and staining same with Geimsa Romanosky stain (Puchkov, 1964). The stained blood was left for 25 minutes for the Geimsa stain to act on it. Thereafter the stained blood was flooded with distilled water and rocked gently to evenly mix the distilled water and stain. The stained blood was washed with water and allowed to dry. The slide was viewed with a binocular microscope to identify the leukocyte species which was calculated as a percentage.

2.5 Biochemical analyses

The blood serum was obtained by centrifuging the blood sample at 5 000 rpm for five minutes and the protein in the samples was spectrophotometrically determined using Biuret method (Gornall *et al*., 1949) at 540 nm. The blood glucose was determined by Roe (1955) method and the cholesterol concentration was determined by the method of Abell and Levy (1952).

2.6 Statistical Analysis

The data was statistical analyzed using one way analysis of variance (ANOVA) followed by LSD post hoc test at 95% confidence interval (Steel and Torrie,1960)

3 RESULT

3.1 Leucocytic Response

The changes in the total leucocyte and differential white blood cell count in *C. gariepinus* exposed to sublethal lead chloride are presented in Table 1. The leucocyte count in the control group did not vary ($p < 0.05$) during the study. The leucocyte count increased with concentrations and when compared with the control, there was significant ($p < 0.05$) leucocytosis in the treatment groups which was both concentration and duration dependent. Five different subspecies of leucocytes (lymphocytes, monocytes, neutrophils, eosinophils and basophils) were identified in the fish during the study. The lymphocyte and the monocytes constituted the agranulocytes identified while neutrophil, eosinophils and basophils were the granulocytes recorded due to the presence of granules in their cytoplasm.

The lymphocytes were the most abundant leucocyte group identified in the blood of the fish exposed to lead. Both small and large lymphocytes were found during the study and they accounted for more than 80 % of the white blood cells. The lymphocytes increased significantly ($p < 0.05$) in the lead-exposed fish when compared with the control. The lymphocytes were significantly different ($p> 0.05$) in the treatment groups and the lymphocytosis was both concentration and duration dependent. The monocytes decreased in the lead-exposed fish on the 7^{th} day and thereafter, it increased significantly in the treatment groups ($p <0.05$) when compared with the control. The basophils were the most abundant granular leucocyte in the peripheral blood of *C. gariepinus* exposed to lead. The proportion was highest in the first week and on day 21 of the study. The neutrophils are the second largest granular white blood cell in the fish while the eosinophils are the least abundant subpopulation.

3.2 Effect on serum glucose

The changes in the serum glucose concentration in *C. gariepinus* exposed to lead are shown in Fig.1. The glucose concentration in the control did not vary throughout the study. The serum glucose level on day 7 were 26.50 ± 2.12 g/dl and 30.50 ± 0.70 g/dl in the fish exposed to 0.1 and 0.4 mg/L lead, respectively. On day 28, the serum glucose concentrations were 52.50 ± 2.12 and 70.00 ± 2.83 g/dl in the groups exposed to 0.1 and 0.4 mg/l lead, respectively. There was concentration and duration significant increase ($p < 0.05$) in the treatment groups when compared with the control and the values differed also in the treatment groups ($p < 0.05$) at each sampling period. The percentage increase in the serum glucose levels were 3.92 and 19.6 in the fish exposed to 0.10 and 0.40mg/L lead after one week, respectively. At the end of the study it increased by 107.5 % and 176.6% in the group treated with 0.10and 0.40mg/L Pb, respectively.

3.3 Effect on serum protein

The result showed that the protein level in the control did not vary ($p > 0.05$) throughout the study while the serum protein in the lead – exposed fish did not differ ($p > 0.05$) from the control value during the first 14 days (Fig 2). Generally, there was progressive concentration and duration dependent increase in the serum protein in the lead–exposed fish as it increased from 4.06±0.06mg/dl on day 7 to 5.28±0.05 mg/dl on day 28 in the fish exposed to 0.1mg/L lead, representing 7.1% increase. When the fish treated with 0.4mg/L lead, the serum protein increased from 4.54±0.37 mg/dl on day 7 to 6.38±0.19 mg/dl on day 28, respectively, representing 29 % increase. Statistical analysis showed that serum protein levels in the treatment groups differed significantly ($p <0.05$) at the end of the study.

3.4 Effect on serum cholesterol

The serum cholesterol level in the control did not vary ($p < 0.05$) throughout the study (Fig. 3). When compared with the control, the cholesterol concentration was significantly higher ($P < 0.05$) in the lead-exposed fish. Also, the cholesterol level differed significantly ($p < 0.05$) in the treatment groups throughout the study. The cholesterol concentration increased from 122.5±3.53 mg/dl on day 7 to 214.0 ± 1.80 mg/dl on day 28 in the group exposed to 0.10mg/L Pb, representing 79.8% increase. When the fish was exposed to 0.40mg/L Pb, the cholesterol concentration increased from 152.5 ±3.54 in the first week to 308± 5.19mg/dl on 28th day of the study, representing 158% increase.

4 DISCUSSION

The result of the study shows that lead has effect on the leucocyte count of *C. gariepinus* and it is in agreement with the observation of Ruparella *et al* (1990) that in *O. mossambicus* exposed to 0.1-10.0 µg/L Cd, the small lymphocytes decreased in number while the neutrophils increased. Similar trend was observed in *Cyprinus carpio* treated with 140mg/L Zn for 96h (Svobodova *et al*, 1994). Besides increased leucocyte count in *Anabas testudineus* exposed to mercury, Kumar et al (2004) the fish also experienced neutrophilia, monocytosis and eosinophilia. Lymphocytosis was reported in fish exposed to treated paper and pulp mill effluents as well as DHAA (Landman *et al*. 2006). These authors further reported deceased number of granulocytes in the fish. On the contrary, Annune and Ahuma (1998) reported decreased leucocyte count in *Clarias gariepinus* exposed to 0.45mg/L Pb for 4 to 5 days.

The general leucocytosis reported in this study is consistent with the observation of Tort and Heramdez-Pascual (1990) in the dogfish exposed to 50µg/L Cd for 4 days.

The observed increase in the serum glucose level in *C. gariepinus* exposed to lead in this study is in accord with report of some earlier workers. Increased serum glucose has been reported in several species like in *Ictalurus nebulosus* (Christensen *et al* 1972) and in *Salmo gairdneri* (Lauren & McDonald, 1985) exposed to metals. Similar increase in plasma glucose was also reported in *Prochidolus lineatus* exposed to lead (Martinez *et al.,* 2004) and in *Oreochromis niloticus* exposed to copper (Monteiro *et al.,* 2005). Our report is in agreement with the reported increase in plasma glucose in trout exposed to pulp paper mill effluent and dehydroabietic acid (Landman *et. al.*, 2006) and in *Cyprinus carpio* exposed to diluted sewage (Kakuta *et al*., 1994) as well as in *Mugil* and *Salmo gairdneri* exposed to either copper and cadmium (Emad *et al*, 2005; Larsson and Haux, 1984). It has been widely reported that hyperglycaemia in fish arises due to the stimulation of catecholamines and corticosteroids (Brown, 1993). The increased serum glucose level in this study is an evidence of stress due to lead exposure as Ozgur and Kargm (2010) argued that coping with such stress is an energy demanding process that requires the fish to mobilize metabolically energy substrates through intense gluconeogenesis. Glucose being one of such known substrates is mobilized through gluconeogenesis to meet this challenge (Vosyliene, 1999). Heavy metals have been reported to act antagonistically with glucocorticoids by inhibiting the receptors thereby disrupting the osmotic and mineral regulatory mechanisms (Wendelaar-Bonga, 1997). Serum increase in glucose due to toxicants has have been associated with hypothalamus-sympathetic-chromaffin cells (McDonald & Milligan, 1997) instead of the hypothalamus-pituitary-interenal axis (Arends *et al* 1999) that is known to have tremendous influence on carbohydrate metabolism.

The result of this study showed that the serum total protein was increased in *C. gariepinus* exposed to

lead. Similar increase in the serum protein level was reported in *Oreochromis niloticus* exposed to metals (Ozgur & Kargm, 2010). Also increased plasma protein was reported in *Mugil* exposed to 0.5ppm copper and cadmium (Emad *et al*, 2005). On the contrary, decreased tissue protein was reported in *Oreochromis niloticus* treated with cadmium (Almeida *et al.,* 2001) and in C*yprinus carpio* when exposed to heavy metals (Vinodhini and Narayanan (2008).

Since the serum proteins are mobilized from the liver, the observed increase in the serum protein in *Clarias gariepinus* exposed to lead could partly be due to enhanced protein production in the liver following exposure to lead. It is known facts that metal are known to affect the gill epithelium with consequent effect on the plasma osmolarity that impair gill exchange capacity. Thus, the elevated serum protein in this study could further be explained partly by this altered plasma osmolarity that favours protein concentration in the serum. Furthermore, it could be due to changes in enzyme activities that favour the formation of soluble protein for possible energy utilization (gluconeogenesis) and for the rapid transport of metal ions to the kidney or liver for elimination as metallothionien.

The enhanced serum cholesterol in this study is an indication of hypercholesteremia in the fish due to the stimulatory effect on the cholesterol biosynthetic pathway. Reduced serum cholesterol level has been reported in *Oreochromis* exposed to some heavy metals (Ruparelia *et al*, 1989; Ozgur & Kargm 2010) and in *Lepomis macrochirus* exposed to methyl mercuric chloride (Dutta & Haghighi, 1986). Decreased cholesterol level was also reported in *Oreochromis niloticus* exposed to heavy metals (Ozgur and Kargm, 2010).The observed elevated cholesterol in this study could have resulted in part to the adverse effect of lead on the liver leading to altered cholesterol metabolism resulting in increased serum cholesterol. This according to Oner *et al* (2008) could be due to liver and kidney failure that resulted in the release of cholesterol into the blood stream.

Conclusion

Generally, the leucocytosis observed in this study gives indication that exposing the fish to lead predisposes it to secondary infections. Also, the reported hyperglycaemia, increased serum protein and cholesterol levels are indications of altered carbohydrate, lipid and protein metabolism in the fish due to lead exposure. Serum parameters which provide information as to state of the internal environment of the fish are known to respond quickly to changes in the water quality. The changes in these biomarkers are a reflection of organ dysfunction in the fish due to metal exposure and these biomarkers could be used in ecotoxicological assessment and as early warning indicators of pollution.

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Table 1: Effect of lead chloride on the differential white blood cell count of *C. gariepinus*

Leucocyte species	Concentration (mg/L)	Duration(days)			
		7	14	21	28
Lymphocyte	**Control**	81.00±1.41^{a1}	80.50±3.54^{a1}	80.00±1.41^{a1}	85.50±0.71^{a1}
	0.10	83.50±2.12^{b1}	84.50±0.71^{b2}	85.50±2.12^{b3}	87.50±3.54^{b4}
	0.40	83.50±2.12^{b1}	86.00±2.83^{b2}	87.00±1.41^{c3}	87.00±1.41^{b4}
Monocyte (%)	**Control**	2.50±0.71^{a1}	2.30±1.41^{a1}	2.40±0.71^{a1}	2.50±0.71^{a1}
	0.10	1.00±1.41^{b1}	5.50±1.41^{b2}	6.00±1.41^{b2}	4.00±0.00^{b2}
	0.40	0.50±0.71^{c1}	6.00±2.83^{c2}	5.00±1.41^{c3}	5.50±1.41^{c3}
Neutrophil (%)	**Control**	1.50±0.71^{a1}	1.50±0.71^{a1}	1.50±0.71^{a1}	1.30±0.71^{a1}
	0.10	1.00±1.41^{b1}	2.00±1.41^{b2}	1.50±0.71^{a3}	1.50±0.71^{a3}
	0.40	0.50±0.71^{c1}	1.50±0.71^{c2}	0.50±0.71^{b} 1	2.00±1.41^{b3}
Eosinphil (%)	**Control**	0.50±0.71^{a1}	0.50±0.71^{a1}	0.60±0.71^{a1}	0.50±0.71^{a1}
	0.10	0.50±0.71^{a1}	1.00±1.41^{b2}	0.50±0.71^{a1}	-
	0.40	-	0.50±0.71^{a1}	0.50±0.71^{a1}	0.50±0.71^{a1}
Basophil (%)	**Control**	10.50±0.71^{a1}	10.50±2.12^{a1}	9.80±2.12^{a1}	10.50±2.12^{a1}
	0.10	15.50±0.71^{a1}	5.50±0.71^{b2}	8.50±0.71^{b2}	7.00±2.83^{b3}
	0.40	15.50±0.71^{a1}	6.00±2.83^{c2}	7.00±4.24^{c3}	7.00±1.41^{b3}
Leucocyte (x10^4/mm^3	**Control**	2.47±0.08^{a1}	2.50±0.02^{a1}	2.49±0.16^{a1}	2.47±0.04^{a1}
	0.10	2.54±0.04^{b1}	3.38±0.60^{b2}	4.28±0.11^{b3}	4.84±0.01^{b4}
	0.40	2.94±0.10^{b1}	3.96±0.06^{c2}	4.55±0.19^{c3}	5.55±0.04^{c4}

Value in the same column with the same superscript (lower case) are not significantly different (p = 0.05) between different concentrations within the same exposure duration. Values with different numeric superscripts differ significantly (P=0.05) between different exposure periods within the same concentration.

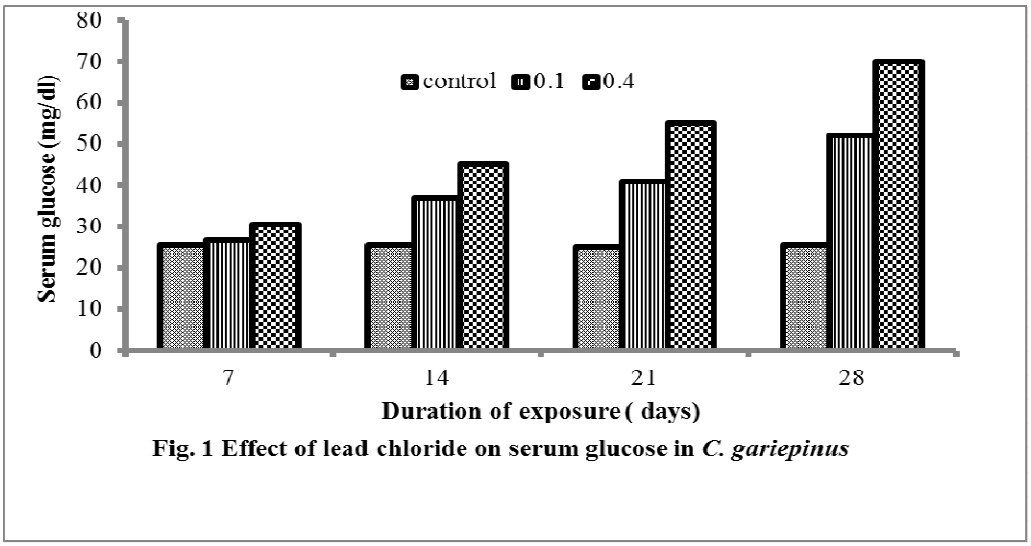

Fig. 1 Effect of lead chloride on serum glucose in *C. gariepinus*

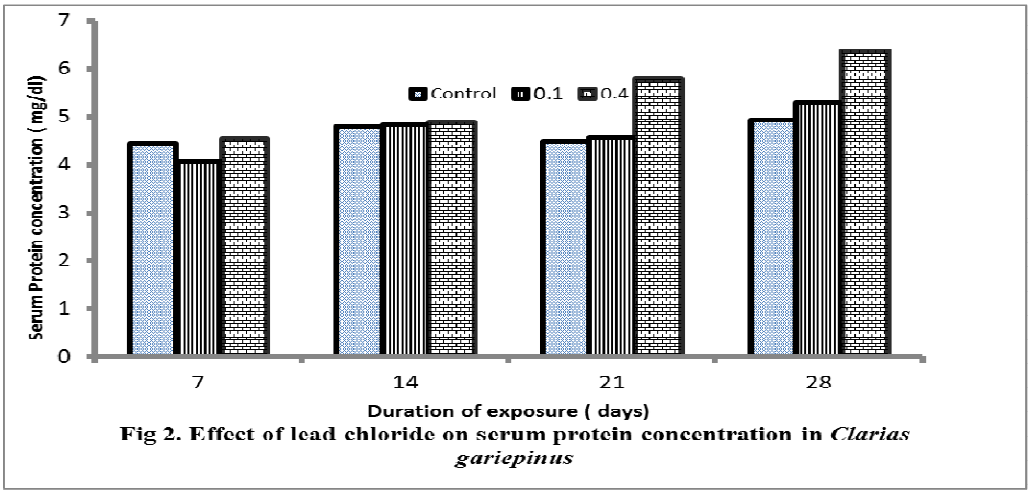

Fig 2. Effect of lead chloride on serum protein concentration in *Clarias gariepinus*

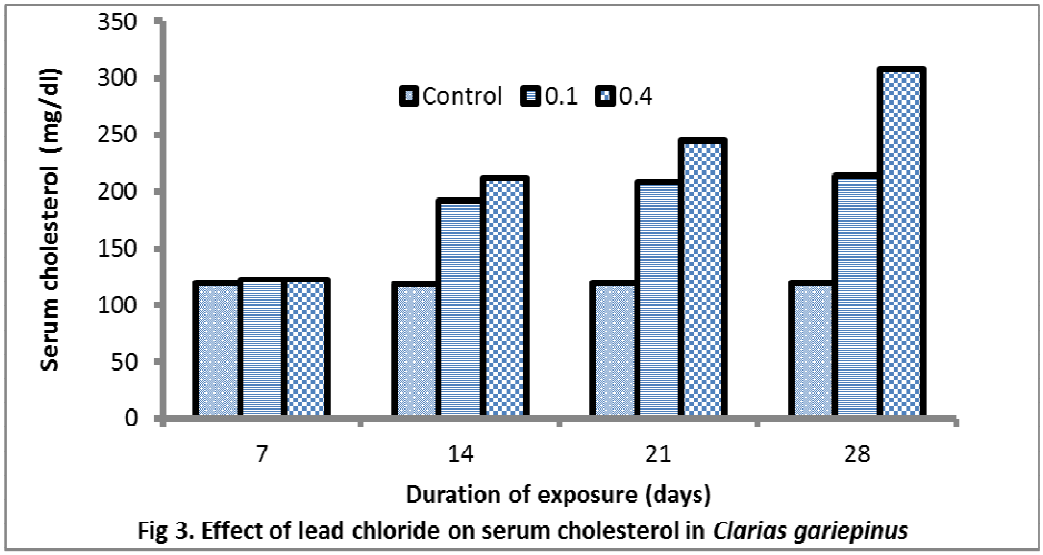

Fig 3. Effect of lead chloride on serum cholesterol in *Clarias gariepinus*

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