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Sub-chronic Exposure to Magnesium Hydroxide Nanoparticles Alters Some Biochemical Parameters in *Clarias gariepinus*

Bukola T. Aluko[1*], Omotade I. Oloyede[1], Ebenezer T. Adebayo[2], Adejoke A. Adewumi[3], Elijah O. Odesanmi[1], Yemisi R. Alli Smith[1], Oladimeji S. Tugbobo[4]

[1]Department of Biochemistry, Faculty of Science, Ekiti State University, Ado Ekiti, Nigeria
[2]Department of Biological Sciences, University of Medical Sciences, Ondo, Nigeria
[3]Department of Zoology and Environmental Biology, Faculty of Science, Ekiti State University, Ado Ekiti, Nigeria
[4]Department of Science Technology, Biochemistry Unit, Federal Polytechnic, Ado Ekiti, Ekiti State, Nigeria

[*]Corresponding Author: bukola.aluko@eksu.edu.ng ; bukola.aluko3@gmail.com

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ABSTRACT

Magnesium hydroxide nanoparticles (MgOHNPs) have gained commercial interest in the areas of waste remediation and recycling of wastewater for portable use. MgOHNPs is among the least investigated nanoparticle and thus its toxicological effects are yet to be unveiled. *Clarias gariepinus* were exposed to 62.5, 125, and 250 mg/L suspensions of small size (10 nm) MgOHNPs for 21 days under semi-static conditions. Thereafter, they were sacrificed and alterations in the hematological parameters, and some biochemical indices were examined. The nanoparticle was characterized by UV/Vis spectrophotometry, and Fourier transform infrared spectroscopy (FTIR). The water quality parameters such as chloride ions and dissolved oxygen significantly decreased, while pH, total alkalinity, carbon (IV) oxide, total hardness, and nitrate increased significantly in the exposed media compared to the control medium. The results of this investigation revealed a concentration-dependent increase in the plasma levels of albumin, total protein, magnesium, calcium, chloride, alkaline phosphatase, and acid phosphatase. Whereas, the levels of aspartate aminotransferase, alanine aminotransferase, glucose, total bilirubin, potassium, and creatinine decreased significantly ($P < 0.05$). The hematological parameters remained unaltered in the fish at the tested doses. The results indicated that exposure to MgOHNPs could lead to disturbances in blood biochemistry of *C. gariepinus*

INTRODUCTION

Nanoparticles are solid colloidal particles ranging in size from 1nm - 100nm. They consist of macromolecular materials in which the active principle is dissolved, entrapped or encapsulated. Their shapes can be spherical, cylindrical, tubular, conical, hollow core, spiral, flat, etc. or irregular (Machado *et al.*, 2015). Nanoparticles can be prepared from a variety of

materials such as proteins, polysaccharides and synthetic polymers. Nanoparticles can be formulated, as injections consisting of spherical amorphous particles which can be safely administered by the intravenous route (Mohanraj and Chen 2006).

Magnesium nanoparticles are relatively non-toxic and odorless white powder with high melting point and hardness. Nanoparticles are widely used in industries due to their biocompatibility, biodegradability, and relatively low cost (Aluko *et al.,* 2018). Magnesium nanoparticles have been employed for various medical applications. These include: alleviation of heartburn, stomach sore, and bone regeneration (Bertinetti *et al.,* 2009; Martinez-Boubeta *et al.,* 2010). MgOHNPs have been used in treatment of cancer (Krishnamoorthy *et al.,* 2012) and as antimicrobial (Koper *et al.,* 2002). It has also been used tremendously for the recycling of waste water for portable use (Aluko *et al.,* 2017). The exponentially growing interest in nanoparticles (NPs) for various applications has been adduced to their exceptionally unique properties which differentiate them from bulk materials (Remya *et al.,* 2015). The widespread applications of NPs have raised concerns regarding their negative impact on the ecosystem. The aquatic environment is vulnerable to contamination from engineered nanomaterials (Sun *et al.,* 2014).

Nanotoxicity is a major adverse effect caused by NPs via their interaction with the environment and living systems. The exposure of NPs to living organisms inflicts various types of toxic impairments which include: cytotoxicity, genotoxicity, and epigeneticity (Jennifer and Maciej, 2013). Alterations in biochemical and hematological profiles of an organism are considered to be the potential biomarkers for evaluating the toxicity. Previous literatures have reported fish as an appropriate biological model to examine the toxicity induced by NPs (Griffitt *et al.,* 2007; Verma *et al.,* 2018).

Clarias gariepinus, was selected as an experimental model due to its great aquaculture and commercial value in Nigeria. The findings of this research will provide useful insight into potential toxicities of magnesium hydroxide nanoparticles on the fish when used for water treatment.

MATERIALS AND METHODS

Reagents and Assay Kits

Magnesium hydroxide ($Mg(OH)_2$ nanoparticles, 99% purity, 10nm size) were obtained from US Research Nanomaterials, Inc. 3302 Twig Leaf Lane Houston, TX 77084, USA. The assay kits for creatinine, calcium, sodium, potassium, chloride, magnesium, albumin, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) alkaline phosphatase (ALP), acid phosphatase (ACP), were purchased from Randox Laboratories United Kingdom. All other reagents used were of analytical grade.

Experimental design

One hundred (100) young adult *C. gariepinus* of average length 24–30 cm and weight 125-170 g were collected from local fish pond in Ado Ekiti, Nigeria and maintained in the aquarium separately. Five fish were placed in each of 20 identical, 40-litre-capacity aquaria,

each containing 30 litres of water. The experimental aquaria were aerated and test media were replaced every day using a siphon. The normal illumination (approximately 12 h light and 12 h dark) was maintained throughout the experimental period. The fish were fed 6 g of Coppens grower's feed containing 45% protein three times per day. Dead fish were removed as soon as possible to avoid contamination from decomposition. Acute toxic effect of MgOHNPs was investigated on *C. gariepinus.* All experiments were carried out for a period of 7 days and lethal concentration (LC50) was determined with five different concentrations (62.5, 125, 250, 500 and 1000 mg/L) of MgOHNPs. Among these concentrations, mortality rate was found at two concentrations (500 and 1000 mg/L). Hence, 250 mg/L concentration was used as a maximum value for further experimental studies. For sub-acute toxicity tests, 0, 62.5, 125 and 250 mg/L were introduced to the fish. Groups of five fish were placed in graded nanoparticle concentrations immediately after preparation. Each concentration was replicated in three different aquaria. After 21 days of exposure, the blood samples were collected. Thereafter, the experimental fishes were sacrificed. The muscle, gill and liver were dissected out for further biochemical analysis.

Hematological analysis

Hematological analysis was done according to the method of Alkaladi *et al.* (2015). The parameters analyzed included red blood cells, (RBCs), hemoglobin (Hb), packed cell volume (PCV), white blood cell (WBC), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH).

Biochemical Assays

The nanoparticle was characterized by UV/Vis spectrophotometry and fourier transform infrared spectroscopy (FTIR). Water analysis was done using a pre – calibrated fresh water aquaculture test kit, (Model: Lamotte Aq-2. Code: 3633-03) as described by Ogbuagu *et al.* (2015). The biochemical parameters were determined colorimetrically by employing the standard ready-to-use Randox kits. The parameters assayed for include aspartate aminotransferase (AST), alanine aminotransferase (ALT) alkaline phosphatase (ALP), acid phosphatase (ACP), albumin, total bilirubin, crea tinine, and electrolytes (sodium, potassium, magnesium, and chloride).The manufacturer's instructions for each biochemical parameter were strictly adhered to in the course of the investigations. While total protein levels were assayed as described by Gornal *et al.* (1949). The glucose concentration of the blood samples of the fish was determined with glucose reagent strips using ON CALL PLUS glucometer from ACON laboratories Inc. San Diego USA (Aluko *et al.,* 2017).

Statistical analysis

Experimental data were expressed as mean ± standard deviation (SD) and subjected to one – way analysis of variance (ANOVA) followed by Duncan multiple range test. Values were considered statistically significant at $P < 0.05$.

RESULTS

The aqueous solution of magnesium hydroxide nanoparticles had a characteristic whitish colour. It had a maximal UV absorption at 206 nm with diameter size of 10 nm (Figure 1). Also, the FTIR spectral of magnesium hydroxide nanoparticles is shown in Figure 2.

The physico-chemical parameters of water for the different concentrations of nanoparticle and control media to which the test *C. gariepinus* were exposed over of period of 21 days are presented in Table 1. Mean values of the water temperature were not significantly ($P < 0.05$) affected by the concentrations of MgOHNPs. On the other hand, chloride ions and dissolved oxygen significantly ($P < 0.05$) decreased with increasing concentrations of nanoparticle. However, the values of pH, ammonia, nitrate, alkalinity, carbon (IV) oxide and total hardness in the exposed media significantly ($P < 0.05$) increased when compared to the control value.

The body weight and length of control *C. gariepinus* and those exposed to MgOHNPs are presented in Figures 3 and 4. There was significant ($P < 0.05$) weight and length gain in the control and nanoparticle treated groups.

The effect of different concentrations of nanoparticle on the haematological profile of *C. gariepinus* is shown in Table 2. The results indicated that there was no significant ($P < 0.05$) difference in all the parameters tested when compared with control values.

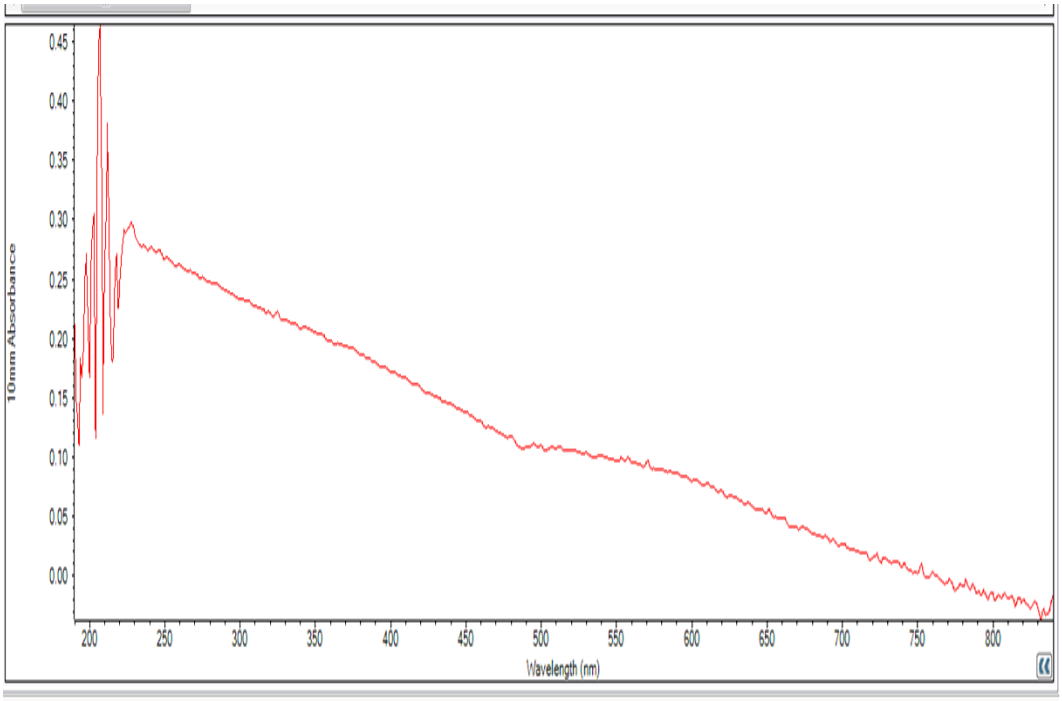

Figure 1: UV/Vis spectrum of magnesium hydroxide nanoparticles

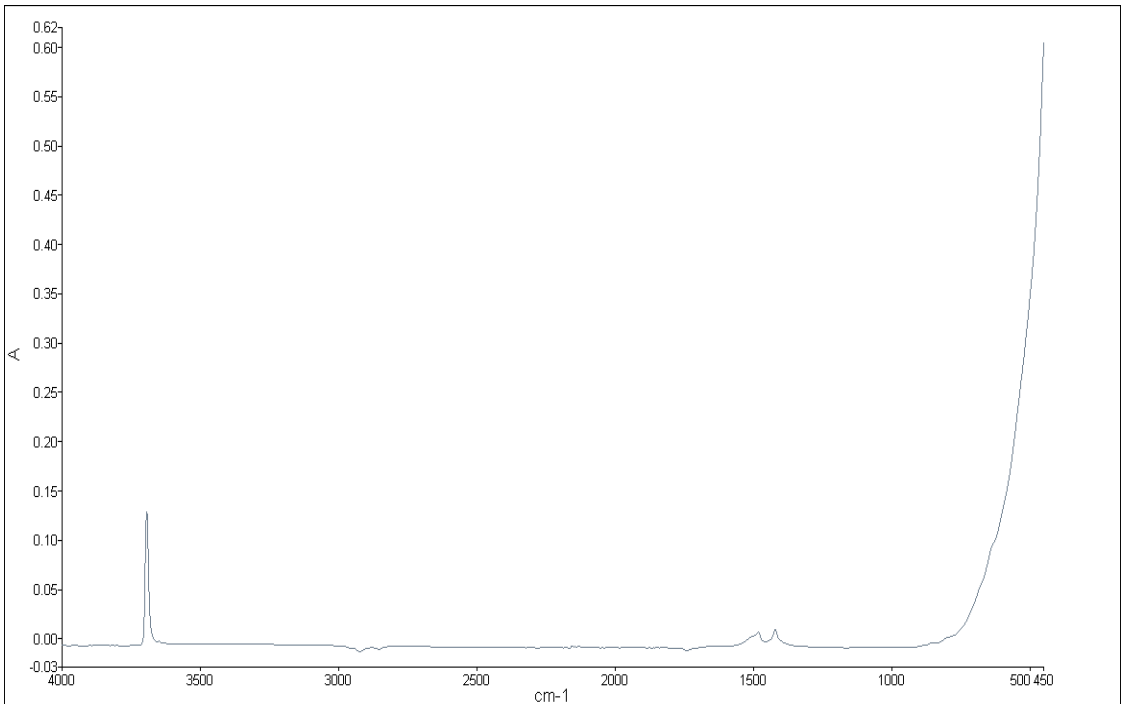

Figure 2: Fourier transform infrared spectroscopy (FTIR) characterization of magnesium hydroxide nanoparticles

Table 1: **Physicochemical characteristics of water before and after exposure to magnesium hydroxide nanoparticles**

Parameter	**Before the Experiment**	**After the Experiment**			
		Control	**2.5mg/L**	**5.0 mg/L**	**10.0 mg/L**
Temperature (°C)	27.0	25.0	25.0	25.0	25.0
pH	5.80	6.8	6.6	6.5	6.3
Ammonia Nitrate (ppm)	0.2	0.25	0.36	0.39	0.44
Nitrate (ppm)	0.03	0.03	0.03	0.06	0.07
Alkalinity (ppm)	24.00	182.0	222.0	238.0	214.0
Carbon (IV) Oxide (ppm)	44.00	58.0	63.5	65.0	71.0
Chloride ions (ppm)	14.00	9.0	11.0	10.0	10.0
Total hardness (ppm)	28.00	44.0	50.0	52.0	60.0
Dissolved oxygen (ppm)	4.30	1.2	0.5	0.42	0.38

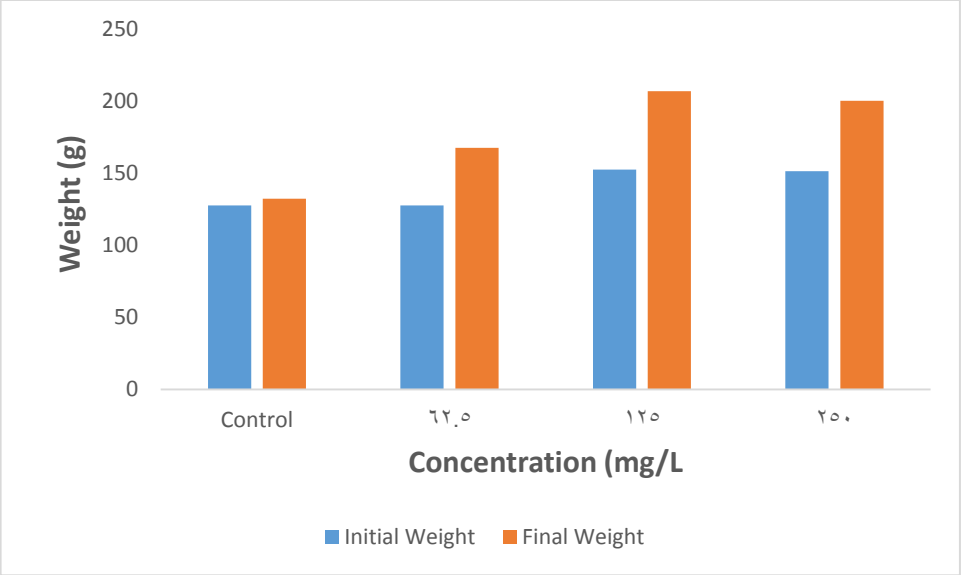

Figure 3: Weight *C. gariepinus* of before and after exposure to magnesium hydroxide nanoparticles

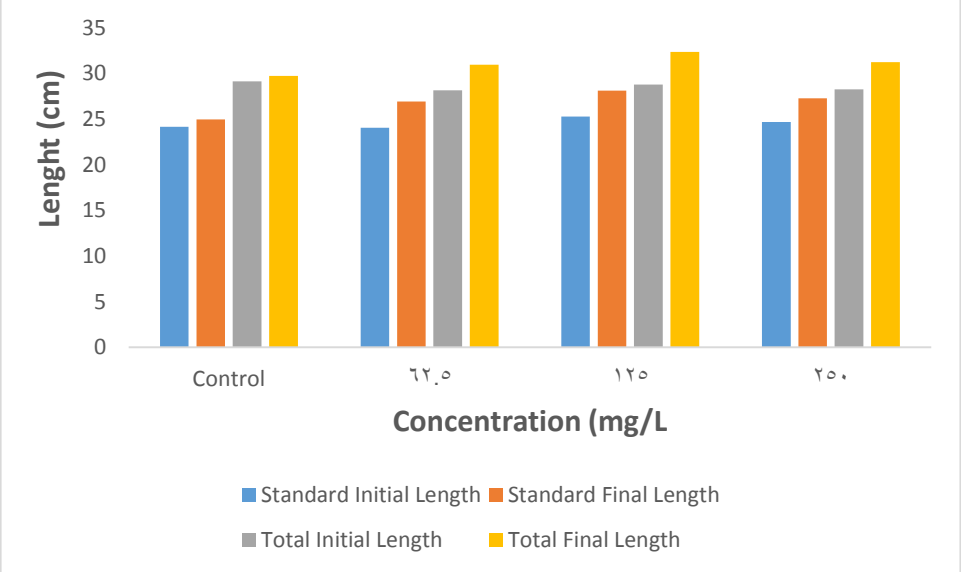

Figure 3: Effect of sub–chronic exposure of magnesium hydroxide nanoparticles on the length of *C. gariepinus*

The effect of sub-chronic exposure of *C. gariepinus* to magnesium hydroxide nanoparticles on some biochemical parameters is shown on Table 3. The observations indicated elevated levels of albumin, total protein, ACP, ALP, magnesium, calcium and chloride. There was a significant ($P < 0.05$) reduction in the values of glucose, total bilirubin and creatinine when compared with the control. However the levels of potassium, AST and ALT remained unaltered throughout the experimental period. Also, a significant ($P < 0.05$) reduction was observed the levels of ALP and total protein in the muscle, liver and gills of *C. gariepinus* exposed to MgOHNPs. However, ALT and AST levels were not altered throughout the period of the experiment.

Table 2: H**eamatological parameters of *C. gariepinus* exposed to magnesium hydroxide nanoparticles for 21 days**

Dose(mg/L)	PCV(%)	HB(g/dL)	RBC(x10^6/L)	WBC(x10^{3}µ/L)	MCV (fL)	MCH (pg)
Control	36.00±6.26^a	16.58±2.80^a	11.48±4.19^a	7.63±1.05^a	33.00±6.50^a	15.13±2.67^a
62.5	40.00±8.34^a	18.32±5.07^a	11.00±4.41^a	8.20±1.04^a	38.08±6.55^a	17.39±4.54^a
125	37.00±9.90^a	16.62±3.37^a	9.37±0.64^a	7.80±1.43^a	39.41±10.08^a	17.70±3.06^a
250	35.17±1.11^a	15.93±3.83^a	9.50±0.39^a	7.65±0.93^a	36.88±2.93^a	16.72±3.88^a

Results are expressed as means ± SD (n=6). Test values carrying superscripts (a) are not significantly different (P <0.05) from the control

Table 3: Effect of repeated doses of magnesium hydroxide nanoparticles on some biochemical parameters in the plasma of *C. gariepinus*

Parameter	Control	62.5 mg/L	125 mg/L	250 mg/L
Albumin (mmol/L)	15.23 ± 4.77^a	19.80 ± 0.97^a	34.67 ± 10.02^b	37.00 ± 5.13^b
Total protein (g/L)	17.46 ± 5.63^a	42.08 ± 6.37^c	38.08 ± 29.40^{bc}	25.62 ± 12.73 ^{b}
Glucose (mmol/L)	7.40 ± 4.33^a	4.07 ± 1.79^b	3.83 ± 1.42^b	3.70 ± 1.74^b
ACP(U/L)	0.23 ± 0.04^a	0.55 ± 0.06^c	0.37 ± 0.04^b	0.41 ± 0.04^b
ALP(U/L)	20.78 ± 2.10^a	17.16 ± 5.26^a	48.71 ± 4.40^b	133.80 ± 6.52^c
ALT(U/L)	2.40 ± 090^a	3.13 ± 0.90^a	3.54 ± 1.41^a	3.12 ± 1.40^a
AST(U/L)	2.25 ± 3.32^a	1.52 ± 0.44^a	0.87 ± 0.16^a	0.90 ± 0.09^a
Magnesium (mmol/L)	1.31 ± 0.50^a	1.72 ± 0.50^{ab}	1.65 ± 0.52^{ab}	2.10 ± 0.27^b
Calcium (mmol/L)	2.47 ± 0.70^a	6.10±2.73^b	5.55±2.20^b	7.63±2.62^b
Potassium (mmol/L)	5.08 ± 1.82^a	3.99 ± 1.07^a	3.77 ± 1.19^a	4.53 ± 1.47^a
Chloride (mmol/L)	76.67 ± 4.37^a	89.06 ± 12.59^b	105.57 ± 3.05^c	106.67 ± 2.89^c
Creatinine (mmol/L)	14.20 ± 1.75^a	0.55 ± 0.06^c	0.37 ± 0.04^b	0.41 ± 0.04^b
Total Bilirubin (µmol/L	7.58 ± 3.08^a	3.26 ± 1.33^b	2.98 ± 2.02^b	2.75 ± 0.46^b

Results are expressed as means ± SD (n=6). Test values carrying superscripts (b - c) are significantly different (P<0.05) from the control (a) for each parameter.

Table 4: Effect of sub-chronic doses of magnesium hydroxide nanoparticles on some markers in the muscle of *C. gariepinus*

Dose (mg/L)	Total Protein (g/L)	AST (U/L)	ALT (U/L)	ALP (U/L)
Control	6.73 ± 1.03^{a}	1.70 ± 0.379^{a}	1.31 ± 0.87^{a}	34.65 ± 4.79^{a}
62.5	3.20 ± 21.51^{b}	1.52 ± 0.27^{a}	1.21 ± 0.91^{a}	22.57 ± 3.70^{b}
125	2.94 ± 6.20^{b}	1.93 ± 0.94^{a}	1.41 ± 0.98^{a}	15.10 ± 1.68^{c}
250	3.31 ± 13.68^{b}	1.56 ± 0.76^{a}	1.31 ± 0.70^{a}	18.83 ± 4.38^{bc}

Results are expressed as means $\pm$ SD (n=6). Test values carrying superscripts (b - c) are significantly different ($P<0.05$) from the control (a) for each parameter.

Table 5: Effect of sub-chronic exposure of magnesium hydroxide nanoparticles on some markers in the gill of *C. gariepinus*

Dose (mg/L)	Total Protein (g/L)	AST (U/L)	ALT (U/L)	ALP (U/L)
Control	10.50 ± 2.75^{a}	3.56 ± 1.05^{a}	2.40 ± 0.90^{a}	47.99 ± 7.25^{a}
62.5	6.76 ± 2.21^{b}	4.15 ± 0.14^{a}	3.12 ± 0.90^{a}	21.02 ± 20.7^{b}
125	4.80 ± 1.55^{b}	3.38 ± 0.40^{a}	3.54 ± 1.41^{a}	19.38 ± 2.44^{bc}
250	2.44 ± 0.77^{c}	3.10 ± 0.39^{a}	3.12 ± 1.40^{a}	13.44 ± 2.36^{c}

Results are expressed as means $\pm$ SD (n=6). Test values carrying superscripts (b - c) are significantly different ($P<0.05$) from the control (a) for each parameter.

Table 6: Effect of sub-chronic exposure of magnesium hydroxide nanoparticles on some markers in the liver of *C. gariepinus*

Dose (mg/L)	Total Protein (g/L)	AST (U/L)	ALT (U/L)	ALP (U/L)
Control	11.46 ± 4.58^{a}	6.34 ± 0.47^{a}	4.31 ± 1.26^{a}	52.75 ± 3.32^{a}
62.5	10.56 ± 1.72^{a}	5.52 ± 3.09^{a}	2.93 ± 0.32^{a}	32.20 ± 3.44^{b}
125	8.27 ± 2.88^{a}	6.17 ± 2.22^{a}	3.92 ± 0.88^{a}	13.62 ± 1.36^{d}
250	10.13 ± 2.15^{a}	7.53 ± 1.45^{a}	3.93 ± 1.38^{a}	21.96 ± 2.15^{c}

Results are expressed as means $\pm$ SD (n=6). Test values carrying superscripts (b - c) are significantly different ($P<0.05$) from the control (a) for each parameter.

DISCUSSION

The findings of this research show that the water quality parameters were significantly altered by MgOHNPs. It has been reported that water quality largely regulates the distribution and productivity levels of aquatic organisms (Spaak and Bauchrowitz 2010). The observed significant variation in water quality may affect some biochemical reactions and metabolic rates of the test organism (Ogbuagu *et al.*, 2015). The weight and length gain by

the fish during the experimental period may be an indication that the nanoparticle did not affect the growth of the fish (Ezeja et al., 2014).

Hematological parameters are patho-physiological reflectors of the whole body of an organism. They are vital tools in the diagnosis of the structural and functional status of fish exposed to contaminants. The non –significant effect of MgOHNPs on these parameters is an indication that the nanoparticle did not affect the hematopoietic system of the fish at the tested doses (Aluko *et al.,* 2017).

The measurement of serum biochemical parameters is used as important index for the assessment of abnormalities in the functionality of the liver and other tissues (Banaee *et al.,* 2011). Blood glucose has been shown to be a sensitive indicator of environmental stress for any chemical pollutant. Alterations in serum glucose) levels are important parameters used as biomarkers of stress in fish (Kaya *et al.,* 2014). Increase in glucose levels has been attributed to increased glucogenesis and glycogenolysis as well as inhibition of glycogenolysis and glycogenesis during stress (Mostakim *et al.,* 2015). The decrease in value of glucose compared to the control indicated activation of glycogenesis and glycolysis by MgOHNPs. Magnesium ions (Mg^{2+}) regulate the most important glycolytic enzymes by acting as their cofactors such enzymes include: hexokinase, phosphofructokinase, aldolase, phosphoglycerate kinase, and pyruvate kinase (De Baaij *et al.,* 2015).

Alterations in the levels of markers such as ACP, ALP, albumin, total bilirubin and total proteins are associated with hepatic damage. Elevated levels of enzymes in the blood result from leakage from tissues (Pendota *et al.,* 2010). The observed significant increase in the activities of ACP and ALP in the plasma with corresponding decrease in the muscle, gill and liver may be attributed to compromised integrity of the membranes these organs (Aluko *et al.,* 2018). Although, the activities of ALT and AST remained unaltered, the elevation of plasma levels of albumin, total bilirubin and total proteins could be as a result of derangements in the functionality of the liver cells (Adedara *et al.,* 2014). The elevated levels of magnesium, calcium, potassium and chloride may be as a result of impaired renal function (Adeyemi *et al.,* 2015).

CONCLUSION

The results obtained in this study demonstrated that magnesium hydroxide nanoparticles altered the physiochemical property of the water. It also implies that the sub-chronic exposure of *C. gariepinus* to magnesium hydroxide nanoparticles at the tested doses could impose deleterious effects on the tissues of fish which may impair their functionality and eventually cause death. However, further investigations should be done to ascertain the level of accumulation of this nanoparticle in critical organs.

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