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Tissue-specific changes in Ca^{2+}-ATPase and Na^{+}/K^{+}-ATPase activities in freshwater African catfish *Clarias gariepinus* juvenile exposed to oxadiazon

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Abstract

Background: This study investigated the effect of sublethal concentrations (0.0, 0.3, 0.6, and 1.2 $mg{\cdot}L^{-1}$) of herbicide oxadiazon (ODZ) on the activities of Ca^{2+}-ATPase and Na^{+}/K^{+}-ATPase in juvenile *Clarias gariepinus*.

Methods: One hundred eighty juveniles of *Clarias gariepinus* (mean weight 58.88 ± 1.24 g and mean length 22.34 ± 1.52 cm) were randomly divided into four groups and exposed to sublethal concentrations (0.00, 0.3, 0.6, and 1.2 $mg{\cdot}L^{-1}$ ODZ) for 21 days in a static renewal bioassay system in which the herbicide and water were replaced completely every day. The changes in Ca^{2+}- and Na^{+}/K^{+}-ATPase activities in the gill, kidney, liver, and heart of the fish were assayed on days 1, 7, 14, and 21.

Result: The result showed significant alteration in the activity of these membrane-bound enzymes. There was duration and concentration-dependent significant ($p < 0.05$) increase in Ca^{2+}-ATPase activity in the treatment groups when compared with the control. The Na^{+}/K^{+}-ATPase activity was significantly ($p < 0.05$) inhibited in all the tissues when compared with control. The observed alterations in the activities of both Ca^{2+}-ATPase and Na^{+}/K^{+}-ATPase in this study may be indication of impaired ionic transport and imbalance in the fish which may trigger other biochemical, physiological, and even neurological consequences that may compromise several body functions.

Conclusion: The alteration of the ATPase activities in *C. gariepinus* by ODZ is likely to affect the ATP usage and energy metabolism in the fish serious repercussions on Ca^{2+} homeostasis, Na^{+}/K^{+} sodium pump, and Ca^{2+}/Na^{+} exchanger The results suggested that assay of the enzymes could be used as a biomarker of water pollution.

Keywords: *Clarias*, Herbicide, Ca^{2+}-ATPase, Na^{+}/K^{+}-ATPase, Ionic imbalance, Osmoregulation

Background

Herbicides are a group of chemicals specially developed to kill weeds in agricultural farms and latter applied in horticulture. They are primarily designed for use in terrestrial agriculture but may be purposefully applied directly on few occasions, to kill aquatic weeds especially in undrainable ponds. The terrestrially applied herbicides may also eventually reach water bodies as runoff. On reaching the water bodies, they alter the water chemistry with consequential effects on the biochemistry and physiology of non-target fauna (Parthasarathy & Joseph, 2011; Adedeji & Okocha, 2012; Harabawy & Ibrahim, 2014; Nara, 2016). The toxic effect of herbicides to fish depends on the chemistry, solubility in

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water, bioconcentration factors, and persistence in the environment as well as on the metabolites.

Oxadiazon (ODZ), 5-tert-butyl-3-(2,4-dichloro-5-isopropoxyphenyl)-1-3,4-oxadiazol-2-one, was developed primarily for use as a preemergent and early postemergent herbicide against broad-leaved weeds (Garbi, Casasus, Martinez-Alvarez, Robla, & Martin, 2006). The metabolism involves alkaline hydrolysis of the oxadiazole ring (Garrido, Lima, Delerue-Matos, Borges, & Brett, 2001) with the formation of tetrahedral intermediate metabolite following nucleophilic attack on the hydroxide unit of the carboxyl group.

ATPases are membrane-bound enzymes that play a central role in cellular physiology as energy transducers (Temiz, Cogun, & Kargin, 2018; Waugh, 2019). They catalyze the hydrolysis of adenosine triphosphate to adenosine diphosphate (ADP) or adenosine monophosphate (AMP) to liberate inorganic phosphate (Pi) with attendant release of energy require for cellular metabolism. Na^+/K^+-ATPase is an oligomeric enzyme (de Lores Arnaiz & Orderes, 2014) with α- and β-subunits that facilitate active transmembrane movement of Na^+ and K^+ ions and cellular water balance (Lingwood & Harouz, 2006; Lavanya, Kavitha, & Malarvzhi, 2011). Ca^{2+}-ATPase, also called calcium pump, on the other hand, is responsible for the transport of Ca^{2+} across the membrane and ensure intracellular calcium ion homeostasis. The enzyme maintains the huge Ca^{2+} gradient across the membrane with amazing precision.

The clariid catfishes particularly the big *Clarias* species are important food fish in tropical and subtropical Africa and Asia in general and Nigeria in particular. One of the big *Clarias*, *C. gariepinus*, is a choice species for aquaculture industry in Nigeria on the account of being able to withstand stress, and it grows to a big size. The fish accounts for more than 80% of inland fish landing in Nigeria. Presently, there are several studies on the effect of pesticides on the activities of Ca^{2+}-ATPase and Na^+/K^1-ATPase in many fishes and mammalian models (Hazarika, Sarkar, & Kataria, 2001; Lingwood & Harouz, 2006; Parvez, Sayeed, & Raisuddin, 2006; Parthasarathy & Joseph, 2011; de Lores Arnaiz & Orderes, 2014; Gable, Addallah, Najjar, Liu, & Askari, 2014; Nara, 2016; Ajima, Pandey, Kumar, & Poojary, 2018). However, there is currently paucity of literature on the effect of ODZ on *C. gariepinus* especially on membrane-bound enzymes like ATPases. To the best of our knowledge, no studies have been carried out on the effect of ODZ on Ca^{2+}-ATPase and Na^+/K^1-ATPase activities in *C. gariepinus*, a very widely distributed African catfish. This study was therefore undertaken to investigate if sublethal concentrations of ODZ will have any effect on the activities of these membrane-bound enzymes in the fish.

Materials and methods

Handling of the experimental fish

Juveniles ($n = 180$) of *C. gariepinus* (58.88 ± 1.24 g, mean length 22.34 ± 1.52 cm) were bought from a private fish farm, Aquafish Nigeria Ltd, Awka, Anambra State, Nigeria. The fish was transported to our wet laboratory in a plastic fish transport container in the morning (6.30 −10.00 h) to minimize heat stress. On arrival to the laboratory, the fish was treated with 0.5% potassium permanganate solution as prophylaxis for 30 min. Thereafter, the fish was acclimatized for 14 days before the commencement of the study. The physicochemical properties (temperature 26.8–28.4 °C, pH 7.1–8.5; conductivity 253–266 $\mu s \cdot cm^{-1}$; dissolved oxygen 6.5–7.8 $mg \cdot L^{-1}$ $CaCO_3$; hardness 258–271 $mg \cdot L^{-1}$ $CaCO^3$) of the non-chlorinated borehole water were determined using standard methods (APHA, 2005). The fish was fed with Coppens commercial fish feed containing 40% crude protein at 5% body weight during the acclimatization period. The species was verified by Prof. J. E. Eyo of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka using the taxonomic keys developed by Teugels (1986) and Hansens (2009).

Experimental design and exposure to sublethal concentrations of ODZ

The experiment was conducted in a static renewal bioassay system in which the water and the herbicide were changed completely every day based on the Organization for Economic Cooperation and Development (OECD, 1992) guidelines No. 203. One hundred eighty juveniles were randomly divided into four treatment groups of 45 fish each. Each treatment group was further randomized into three replicate experiments of 15 fish per replicate. Each replicate experiment or culture unit consists of a 75-L plastic aquarium containing 50 L of borehole water. Each unit was covered with nylon mosquito netting material to prevent the fish from jumping out. The netting material was held in position with nylon twine. The water in each replicate experiment was aerated continually using air pump. Each aquarium was cleaned every day. After cleaning, the water and the herbicide were removed by siphoning using a plastic tubing material. The water containing the herbicide was drained into an earthen pit to avoid contaminating the environment. The fish were fed once daily after cleaning and changing the water. The fish were fed at 2% body weight with Coppens commercial fish feed containing 40% crude protein. Three sublethal concentrations (0.3, 06, and 1.20 $mg \cdot L^{-1}$) of ODZ were prepared by dilution using commercial preparation of Ronstar that contains 120 $g \cdot L^{-1}$ ODZ as the active ingredient, and it was added to the water. The concentration of ODZ was kept constant by replacing it daily after removing the water. The

sublethal concentrations used were based on the determined median lethal concentration (LC_{50}) of 1.72 mg·L^{-1} (Oluah, 2019). Fish in groups 1, 2, and 3 were exposed to 0.3, 0.6, and 1.2 mg·L^{-1} ODZ, respectively. The fourth group (control) was exposed to non-chlorinated borehole water only. The experiment was carried out in accordance with the guideline of the Ethical and Animal Care Committee, Faculty of Biological Science, University of Nigeria, Nsukka. The fish was allowed to swim in the test concentrations for 21 days. The experimental fish were sampled on days 1, 7, 14, and 21 to determine the effect of exposure to ODZ on the Na^+/K^+-ATPase and Ca^{2+}-ATPase activities in the fish. On each sampling day, two fish from each replicate experiment were taken and anesthetized using MS 222 before killing by inserting a sharp knife through the frontal fontanelle. All the organs were dissected out and rinsed in physiological saline to remove traces of blood. Thereafter the organs were blotted dry using adsorbent paper towel.

Assay of Na^+/K^+-ATPase and Ca^{2+}-ATPase

The Na^+/K^+-ATPase activity was assayed by measuring the amount of inorganic phosphate (Pi) liberated following the hydrolysis of ATP by ATPase as described by Bonting (1970). Ca^{2+}-ATPase activity was determined by the method of Hjerten and Pan (1983). The enzyme activity is expressed as micromole Pi liberated per minute per milligram protein (μmol Pi·min^{-1} mg $protein^{-1}$). The protein concentration was spectrophotometrically determined by the method of Lowry, Rosebrough, Farr, and Randall (1951) using bovine serum albumin as the standard.

Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 20.0 for Windows. The Duncan multiple range post hoc test was used to compare the means. One-way analysis of variance (ANOVA) was used to analyze the difference in the treatment groups and the control. The results are presented as means ± standard deviation. The significant level was 5% probability.

Result

Effect on $Ca2^+$-ATPase activity

The enzyme activity showed both duration- and concentration-dependent increases after day 1 in all the treatment groups and in all the tissues. Ca^{2+}-ATPase activity in the gill (Fig. 1) increased from 0.15 ± 0.01 on day 1 to 0.51 ± 0.01 μmol Pi·min^{-1}·mg $protein^{-1}$ on day 21 in the group exposed to 0.3 mg·L^{-1} ODZ. Similarly, the enzyme activity increased from 0.17 ± 0.005 and 0.16 ± 0.004 μmol Pi·min^{-1}·mg $protein^{-1}$ in the fish exposed to 0.6 and 1.2 mg L^{-1} ODZ on day 1 to 0.73 ± 0.006 and 0.64 ± 0.005 μmol Pi·min^{-1}·mg $protein^{-1}$ on day 21, respectively. The enzyme activity did not vary significantly ($p > 0.05$) in the control group throughout the study and between the treatment groups and the control on day 1. Thereafter, it differed significantly ($p < 0.05$) between the treatment groups. The gill Ca^{2+}-ATPase activity increased by 188, 305, and 255% in the fish treated with 0.3, 0.6, and 1.2 mg·L^{-1} ODZ, respectively, when compared with the control.

In the kidney, the enzyme activity in the control group did not vary ($p > 0.05$) throughout the exposure period. The kidney Ca^{2+}-ATPase activity was significantly higher ($p < 0.05$) in the treatment groups when compared with the control after day 1 (Fig. 2). The enzyme activity did not vary ($p > 0.05$) in the group exposed to 0.3 mg·L^{-1} ODZ between day 7 and day 21 and between the group exposed to 0.3 and 0.6 mg·L^{-1} ODZ between day 7 and day 14. Generally, the kidney Ca^{2+}-ATPase increased by 88, 100, and 153% in the fish exposed to 0.3, 0.6, and 1.2 mg·L^{-1} ODZ, respectively, at the end of the study.

The liver Ca^{2+}-ATPase activity did not change ($p > 0.05$) during the study in the control and between the

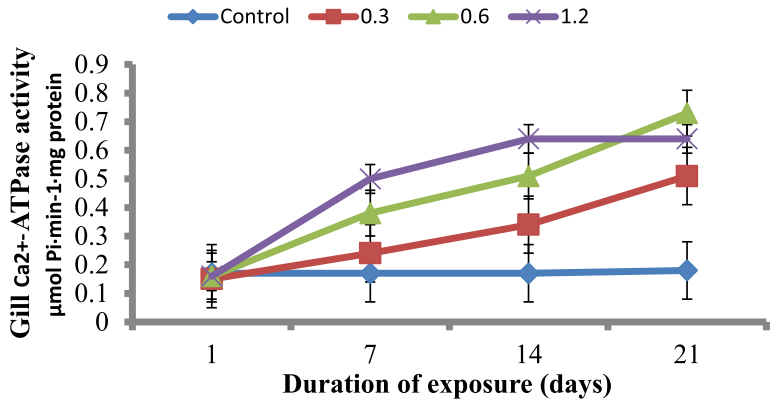

Fig. 1 Changes in gill Ca^{2+}-ATPase activity in *Clarias gariepinus* juvenile exposed to oxadiazon (mean ± SD)

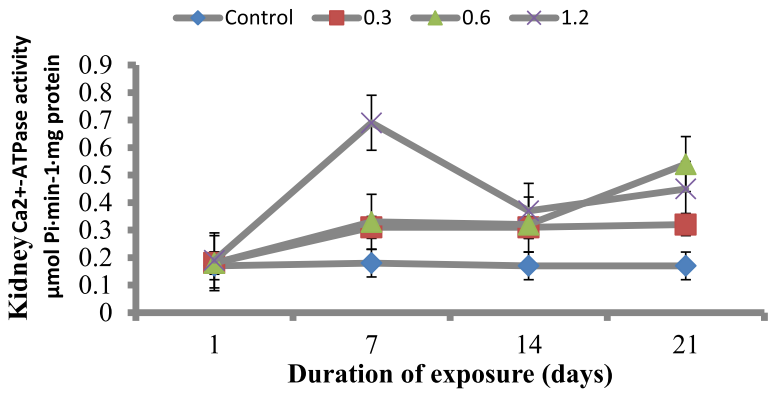

Fig. 2 Changes in the kidney Ca^{2+}-ATPase activity in *Clarias gariepinus* juvenile exposed to oxadiazon (mean ± SD)

treatment groups on day 1 (Fig. 3). As from day 7, the enzyme activity showed significant stimulation in the treatment groups when compared with the control (except in the fish treated with 0.3 mg·L^{-1} ODZ). On day 21, the enzyme activity increased to 0.42 ± 0.11, 0.53 ± 0.12, and 0.63 ± 0.05 µmol·min^{-1}·mg $protein^{-1}$ in the fish exposed to 0.3, 0.6, and 1.2 mg·L^{-1} ODZ, respectively, when compared with the control. This represented 33, 42, and 15% stimulation in enzyme activity in the respective groups.

The heart Ca^{2+}-ATPase activity did not change ($p > 0.05$) during the study in the control, being 0.41 ± 0.08 and 0.48 ± 0.01 µmol Pi·min^{-1}·mg $protein^{-1}$ on day 1 and day 21, respectively (Fig. 4). When compared with the control, the enzyme activity was significantly higher ($p < 0.05$) in the treatment groups, and it also differed ($p < 0.05$) between the treatment groups throughout the study. The ATPase activity was highest within the first 24 h of exposure, and thereafter, the activity decreased progressively with duration in an attempt to approximate the control. In the fish exposed to 0.3, 0.60, and 1.2 mg·L^{-1} ODZ, the enzyme activity increased from 0.41 ± 0.08 µmol Pi·min^{-1} mg $protein^{-1}$ (control) to 1.89 ± 0.01, 2.58 ± 0.15, and 1.56 ± 0.02 µmol·min^{-1}·mg $protein^{-1}$ on day 1, respectively. This represented 360, 285, and 280% increases in the enzyme activity on day 1 in the groups exposed to 0.3, 0.6, and 1.2 mg·L^{-1} ODZ, respectively. The enzyme activity decreased from 1.89 ± 0.01 and 2.58 ± 0.15 µmol·min^{-1}·mg $protein^{-1}$ on day 1 to 0.64 ± 0.01 and 0.68 ± 0.06 µmol min^{-1} mg $protein^{-1}$ on day 21 in the group treated with 0.3 and 0.6 mg·L^{-1} ODZ, respectively. At the end of the study, the heart Ca^{2+}-ATPase activity had increased from 0.48 ± 0.02 (control) to 0.64 ± 0.01, 0.68 ± 0.10, and 0.55 ± 0.06 µmol·min^{-1}·mg $protein^{-1}$ in the fish exposed to 0.3, 0.6, and 1.2 mg·L^{-1} ODZ, respectively. This represented 33, 42, and 14% increases in the enzyme activities in the respective groups.

Effect on the tissue Na^+/K^+-ATPase activity

The effect of ODZ on tissue Na^+/K^+-ATPase activity is shown in Table 1. The Na^+/K^+-ATPase activity was significantly inhibited ($p < 0.05$) in all the tissues and in the treatment groups when compared with the control. The gill Na^+/K^+-ATPase activity was significantly inhibited in the treatment groups following exposure to ODZ when compared with the control. The mean enzyme activity differed ($p < 0.05$) between the treatment groups except

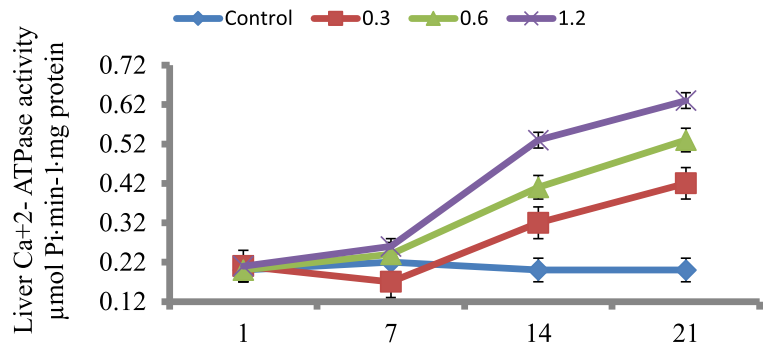

Fig. 3 Changes in the liver Ca^{2+}-ATPase activity in *Clarias gariepinus* juvenile exposed to oxadiazon (mean ± SD)

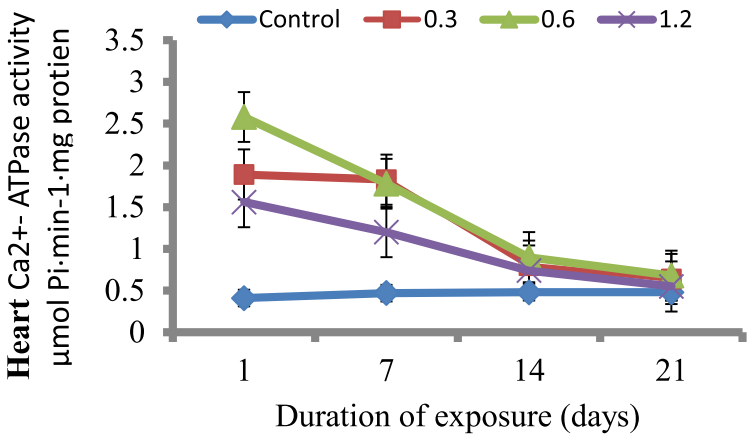

Fig. 4 Changes in the heart Ca^{2+}-ATPase activity in *Clarias gariepinus* juvenile exposed to oxadiazon (mean ± SD)

on day 21 between the fish treated with 0.30 and 0.60 mg·L^{-1} ODZ. The decrease in the gill Na^+/K^+-ATPase activity was concentration and duration dependent ($p < 0.05$). At the end of the study, the percentage enzyme inhibition was 62 in the fish exposed to 0.30 and 0.60 mg·L^{-1} ODZ, while the inhibition was 85% in the fish exposed to 1.20 mg L^{-1} ODZ.

In the kidney, the enzyme inhibition was both concentration and duration dependent. After 24 h, the kidney Na^+/K^+-ATPase decreased form 2.18 ± 0.02 µmol Pi·min^{-1}·mg $protein^{-1}$ in the control to 2.07 ± 0.02 and 1.02 ± 0.05 µmol Pi·min^{-1}·mg $protein^{-1}$ in the fish exposed to 0.30, 0.60, and 1.20 mg·L^{-1} ODZ, respectively. On day 21, the enzyme activity decreased to 2.01 ± 0.01, 0.73 ± 0.09, and 0.44 ± 0.35 µmol Pi·min^{-1}·mg $protein^{-1}$ in the fish exposed to 0.30, 0.60, and 1.20 mg·L^{-1} ODZ, respectively. The mean enzyme activity also differed significantly ($p < 0.05$) between the treatment groups. On day 21, the enzyme activity decreased by 25, 73, and 84% when the fish was exposed to 0.30, 0.60, and 1.20 mg·L^{-1} ODZ, respectively.

The liver Na^+/K^+-ATPase activity did not differ ($p > 0.05$) in the control throughout the study. The mean enzyme activity in the treatment groups differed

Table 1 Changes in the Na^+/K^+-ATPase activity in the tissues of *Clarias gariepinus* exposed to oxadiazon

Tissue	Concentration (mg·L^{-1})	Duration of exposure (days)			
		1	7	14	21
Heart	Control	2.47 ± 0.13^{a1}	2.49 ± 0.07^{a1}	2.43 ± 0.06^{a1}	$2.52 \pm 0.09_{a1}$
	0.3	2.29 ± 0.01^{a1}	1.11 ± 0.03^{b2}	0.84 ± 0.06^{b3}	0.58 ± 0.02^{b4}
	0.6	2.06 ± 0.03^{b1}	1.01 ± 0.01^{b2}	0.67 ± 0.07^{c3}	0.43 ± 0.02^{c4}
	1.2	2.03 ± 0.04^{b1}	0.45 ± 0.01^{c2}	0.23 ± 0.04^{d3}	0.18 ± 0.01^{d4}
Kidney	Control	2.18 ± 0.02^{a1}	2.18 ± 0.02^{a1}	2.16 ± 0.15^{a1}	2.69 ± 0.45^{a1}
	0.3	2.07 ± 0.02^{a1}	2.02 ± 0.33^{a1}	2.10 ± 0.09^{a1}	2.01 ± 0.01^{b1}
	0.6	1.10 ± 0.32^{b1}	1.08 ± 0.01^{b1}	0.91 ± 0.08^{b2}	0.73 ± 0.09^{c3}
	1.2	1.02 ± 0.05^{b1}	0.81 ± 0.07^{c2}	0.63 ± 0.04^{c3}	0.44 ± 0.35^{d4}
Gill	Control	5.34 ± 0.41^{a1}	5.42 ± 0.23^{a1}	5.97 ± 0.37^{a1}	5.47 ± 0.31^{a1}
	0.3	4.69 ± 0.20^{b1}	4.11 ± 0.28^{b2}	$2.71 \pm 0.37^{b\ 3}$	2.04 ± 0.04^{b4}
	0.6	4.30 ± 0.02^{b1}	3.63 ± 0.15^{c2}	2.32 ± 0.45^{c3}	2.04 ± 0.27^{b4}
	1.2	$4.18 \pm\ 0.15^{c1}$	3.06 ± 0.16^{d2}	1.85 ± 0.14^{d3}	0.82 ± 0.17^{c4}
Liver	Control	3.82 ± 0.18^{a1}	3.53 ± 0.15^{a1}	3.50 ± 0.29^{a1}	3.50 ± 0.21^{a1}
	0.3	3.46 ± 0.07^{a1}	3.14 ± 0.14^{b2}	2.06 ± 0.05^{b3}	1.73 ± 0.14^{b4}
	0.6	3.24 ± 0.05^{a1}	3.03 ± 0.04^{c2}	1.66 ± 0.47^{c3}	0.71 ± 0.16^{c4}
	1.2	3.18 ± 0.08^{b1}	2.07 ± 0.36^{d2}	$1,59 \pm 0.05^{c3}$	0.42 ± 0.04^{d4}

Na^+/K^+ activity is expressed as micromole Pi per minute per milligram protein. Values = mean ± SD ($n = 3$ determinations).Values in the same column with different superscript letters differ significantly between different concentrations. Values in the row with different numeric superscripts are significantly different with duration within the same concentration; $p < 0.05$

significantly ($p < 0.05$). The mean enzyme activity decreased from 3.82 ± 0.18 μmol Pi min^{-1} mg $protein^{-1}$ in the control to 3.46 ± 0.07, 3.24 ± 0.05, and 3.18 ± 0.08 μmol Pi min^{-1} mg $protein^{-1}$ on day 1 when the fish was exposed to 0.30, 0.60, and 1.20 mg L^{-1} ODZ, respectively. At the end of the study on day 21, the enzyme activity decreased to 1.73 ± 0.14, 0.71 ± 0.16, and 0.42 ± 0.04 μmol Pi·min^{-1} mg $protein^{-1}$ in the fish exposed to 0.30, 0.60, and 1.20 mg·L^{-1} ODZ, respectively. At the termination of the study, the enzyme activity was inhibited by 51, 80, and 88% in the groups exposed to 0.30, 0.60, and 1.20 mg·L^{-1} ODZ, respectively.

At the end of day 1, the heart Na^+/K^+-ATPase activity decreased from 2.47 ± 0.13 μmol Pi min^{-1}·mg $protein^{-1}$ (control) to 2.29 ± 0.01, 2.06 ± 0.03, and 2.03 ± 0.04 μmol Pi·min^{-1}·mg $protein^{-1}$ in the fish exposed to 0.30, 0.6, and 1.2 mg·L^{-1}, respectively. At end of the study, enzyme activity in the heart decreased to 0.58 ± 0.02, 0.42 ± 0.07, and 0.18 ± 0.01 μmol Pi·min^{-1}·mg $protein^{-1}$ in the group exposed to 0.30, 0.60, and 1.20 mg·L^{-1} ODZ, respectively when compared with the control (2.52 ± 0.09 μmol Pi·min^{-1}·mg $protein^{-1}$). The percentage inhibitions of the enzyme activity in these groups were 76, 83, and 92 in the fish treated with 0.30, 0.60, and 1.20 mg·L^{-1} ODZ, respectively. The mean Na^+/K^+-ATPase activity in the different group was significantly different ($p < 0.05$) and showed significant ($p < 0.05$) concentration- and duration-dependent inhibition. The overall order of inhibition in tissue Na^+/K^+-ATPase activity was heart > liver > kidney > gill.

Discussion

The ATPases are membrane-bound enzymes involved in active transport of cations across the cell membranes (Das and Mukherjee, 2003; Temiz et al., 2018; Waugh, 2019) and play a critical role in intracellular and cellular functions in animals. Aside from maintaining the ionic balance, it provides the required energy to transport metabolites. Basically, calcium ion adenosine triphosphatase is a key protein responsible for the transport of calcium ions across the cell membrane so as to sustain intracellular calcium ion homeostasis. It is a membrane lipid-dependent ubiquitous protein (Temiz et al., 2018; Waugh, 2019) whose functionality depends largely on the chemical composition and physiological phase of the lipid bilayer around the protein.

The observed stimulation of Ca^{2+}-ATPase activity in *C. gariepinus* due to ODZ exposure is in consonance with the results of Vargas-Medrano, Sierra-Fonseca, Arellano-Carrillo, and Plenge-Tellechea (2011) which showed that exposure to 0.2 mM of glyphosate and cypermethrin stimulated Ca^{2+}-ATPase activity in vitro by 155 and 111%, respectively, in human erythrocytes. Similar stimulation of calcium ATPase activity by pyrethroid and piperonyl bentoxide was observed in the brain tissues of mammalian models (Kakko, Tornela, & Tahti, 2003; Grosman & Diel, 2005). In his studies with *Clarias batrachus*, Kumar (2010) reported that deltamethrin exposure caused enhancement in the activities of Mg^{2+}- and Ca^{2+}-ATPases in the gills at a low concentration of 0.07 mg·L^{-1}.

Studies by Diaz and Retamal (2018) and Cordero-Morales and Vasquez (2018) showed that acidic phospholipid and long-chain polyunsaturated fatty acids also stimulated Ca^{2+}-ATPase activities in animal tissues. Similarly, Cao et al. (2003) observed that proteins like cytokines and interleukin activate Ca^{2+}-ATPase activities in mammalian models. Thus, it may be plausible to suggest that exposing the fish to ODZ may have elicited the endogenous release of these molecules that promoted the increase in Ca^{2+}-ATPase activity. On the contrary, Ca^{2+}-ATPase activity was inhibited in freshwater fish, *Cirrhinus mrigala* exposed to zinc cyanide (Shwetha, Praveen, & Hosetti, 2012) and in *Oreochromis niloticus* treated with lambda-cyhalothrin (Parthasarathy & Joseph, 2011). According to Sengar et al. (2003), malathion and carbofuran treatments reduced Ca^{2+}-ATPase activity in the brain tissues of zebrafish, *Danio rerio* and in the fish *Heteropneustes fossilis*. Gush, Shukla, and Ray (2014) reported that exposure to carbofuran inhibited the activity of the enzyme in cultured hepatocytes. Also, the enzyme activity was inhibited in the nervous tissues of slug by pesticides (Souza da Silva et al., 2003) and in the erythrocyte plasma membrane of Nile tilapia, *Oreochromis niloticus* exposed to sodium cyanide (Ramzy, 2014) as well as in crab *Oziotelphusa senex senex* exposed to fenvalerate (Panati, Narala, & R., and Venkatramana, R.A.T., 2012). Results from mammalian sentinels showed that Mg^{2+}/Ca^{2+}-ATPase activities were also reduced when exposed to agrochemicals. For example, Mg^{2+}- and Ca^{2+}-ATPase activities were diminished in the brain of a rat model exposed to acephate (Mohiyuddin, Reddy, Kumai, & Doss, 2010), pyrethroids (Antwi & Reddy, 2015), and chlorpyrifos (Mehta, Verma, & Srivastava, 2005). Similarly, Ksheerasagar, Hiremath, and Kaliwal (2011) demonstrated that carbosulfan inhibited liver Mg^{2+}-ATPase in mice. Reduction in the activity of the enzyme was also reported in the skeletal muscle of mice treated with cartap (Liao et al., 2000) or fed with trans-fatty acid (Ren, Liu, Nie, Zhang, & Chen, 2019). A similar effect was observed in *Rana temporaria* exposed to chlorpyrifos and aluminum chloride (Nozdrenko, Abramchuk, Soroca, & Miroshnichenko, 2015; Nozdrenko, Mirochnychenko, Soroca, Korchinska, & Zavodorskiy, 2016).

The process by which ODZ promoted or enhanced the Ca^{2+}-ATPase activity in this study is not clear. Studies by Tahir and Lokhandwala (2001) showed that

xenobiotic exposure activates dopamine D-like receptors that are involved in the modulation of ATPase activities in animals. This was corroborated by the works of Neve, Seamans, and Trantham–Davidson, H. (2004) and Heusinkveld, Van der Berg, and Westerink (2014) which showed that pesticides and xenobiotics activate the dopamine D_1 receptors that are linked to the feedback mechanism that regulate the voltage-gated calcium ion channel via the protein kinase A (PKA)- and protein kinase C (PKC)-mediated protein phosphorylations that stimulate Ca^{+}-ATPase activity unlike the effect of stimulating dopamine D_2 receptors that results in less phosphorylation with inhibitory effect on the voltage-gated calcium channel with attendant decrease in dopaminergic neurotransmission. Pesticide exposure stimulates the activity of adenyl cyclases resulting in higher production of cAMP that promotes phosphorylation of the sensitive sites on the C-terminal of calcium channels thereby increasing the likelihood of opening the calcium channels and stimulating the activity of Ca^{2+}-ATPase activity (Varadi, Mori, Mikala, & Schwrtz, 1995). It is possible that ODZ may have acted in this way to stimulate the activity of cyclases, resulting in increased cyclic adenosine monophosphate production that facilitated the phosphorylation of several receptor sites on the calcium channel to increase the size of the channel thereby stimulating the activity of Ca^{2+}-ATPase. This is in agreement with the earlier report of Souza da Silva et al. (2003) that herbicides affect the transport channels either by altering the enzyme activity or affecting the membrane structure and phospholipid fluidity. The increase in the activity of Ca^{2+}-ATPases in this study may, according to Sen (2011), be due to the stimulation of calcium-binding proteins and the activation of protein kinase A. As a membrane-bound protein, its activity would be influenced by the status of the membrane phospholipid bilayer arrangement and the relative composition of fatty acids that are central in tuning the physicochemical properties and functions of the membrane (Duncan et al., 2017; Gad, 2011; Lee, 2004).

The result of the study shows that ODZ has an inhibitory effect on Na^{+}/K^{+}-ATPase activity on tissue of *C. gariepinus*. The observed decrease in Na^{+}/K^{+}-ATPase in this study is in agreement with the reported inhibition of the enzyme in Nile tilapia, *O. niloticus*, catfish *C. gariepinus*, and *Catla catla* exposed to cyanide-based contaminants (Oseni, 2015; Praveen, Shwetha, & Basaling, 2012; Ramzy, 2014). It is also consistent with the reported decrease in the enzyme activity in *Oreochromis niloticus* exposed to crude oil (Gad, 2011) and in freshwater crab, *Paratelphusa jacquemontii* treated with malathion (Patil, Kadam, & Raut, 2014). Similarly, a dose and time-dependent decrease in Na^{+}/K^{+}-ATPase activity was reported in fish exposed to dithiocarbamate (Srivastava & Singh, 2013) and in *Clarias batrachus* exposed to deltamethrin (Kumar, 2010). The observed reduction in activity of Na^{+}/K^{+}-ATPase in this study is consistent with the observation in *O. niloticus* exposed to lambda-cyhalothrin (Parthasarathy & Joseph, 2011) and chlorantraniliprole (Temiz et al., 2018) as well as in Indian major carp, *Cirrhinus mrigala* exposed to silver nitrate (Sathya, Ramesh, Poopal, & Dinesh, 2012). Inhibited Na^{+}/K^{+}-ATPase activity was also reported in climbing perch, *Anabas testudineus* exposed to naphthalene (Nayak, Raut, & Patnaik, 2019), in *Cyprinus carpio* exposed to diazinon, cypermethrin and phenolic compounds (Balaji, Nachiyappan, & Venugopal, 2015; Oruc & Usta, 2007; Varadarajan, Hari-Sankar, Jose, & Philip, 2014) and in the crustacean, *Hyalella* species, treated with carbofuran and glyphosate-based herbicide Roundup (Dutra, Fernandes, & Oliveira, 2008; Dutra, Fernandes, Failace, & Oliveira, 2011). The studies by Oruc and Usta (2007) further showed that the pesticide diazinon has variable effect on tissue Na^{+}/K^{+}-ATPase activity in *Cyprinus carpio*. For example, they reported that lower concentrations (0.0036 ppb) of diazinon caused elevated Na^{+}/K^{+}-ATPase activity in the gills after 5 days of exposure, whereas a high concentration of 0.018 ppb diazinon inhibited the enzyme activity on day 5 of exposure. They further reported that on day 15 of exposure, muscle and kidney Na^{+}/K^{+}-ATPase activity were inhibited. Similar biphasic effect was found in rats exposed to Aroclor 1254 (Pathak & Kundu, 2013) as the tissue ATPases were inhibited on day 4 but were stimulated with longer periods of exposure for 12 days. Similar inhibition in the enzyme activity was reported by Paulino, Sakuragui, Ramos, Pereria, and Saduskas-Henrique (2013) in the gills of the fish *Astyanax fasciatus* and *Pimelodus maculatus* exposed to organochlorides and heavy metals as well as in human erythrocytes exposed to fluoride (Shashi & Meenakshi, 2015). The enzyme activity was also inhibited in the tissues of *C. batrachus* exposed to carbofuran (Begum, 2011), chlorpyrifos, and monocrotophos (Narra, Rajender, Reddy, Murty, & Begum, 2017). Similar inhibition was observed in *Channa punctatus* exposed to bleached kraft mill effluent (Parvez et al., 2006) and endosulfan (Singh, Upadhyay, Sharma, & Kumar, 2018) and cyfluthrin for 30 days (Singh, Sharma, & Kumar, 2015). Panati et al. (2012) reported that increasing free fatty acids of tissue in the crab *Oziotelphusa senex senex* treated with fenvalerate resulted in decreased Na^{+}/K^{+}-ATPase activity. Studies with other mammalian models (Blasiak, 1996; Mohiyuddin et al., 2010) also showed a dose-dependent decrease in the Na^{+}/K^{+}-ATPase enzyme in

rats exposed to parathion, organophosphorous, and acephate. Similarly, Mg^{2+}-ATPase and Na^+/K^+-ATPase activities were inhibited in rats exposed to carbosulfan (Ksheerasagar et al., 2011) and anilofos (Hazarika et al., 2001).

The inhibition and/or alteration of ATPases by agrochemicals adversely affect the usage of ATP and energy metabolism in animals by affecting the activities of other enzymes to which ATP or ADP has allosteric effects (Unnisa & Devaraj, 2007; Ksheerasagar et al., 2011). The mechanism by which ODZ affected the Na^+/K^+-ATPase activity is not known with certainty. Some studies (Ulbricht, 2005; Hu, Liang, Shi, & Gao, 2008) showed that pesticide exposure affect membrane bound enzymes by causing loss or decreased membrane fluidity by modifying the membrane protein orientation or by changing the lipid–lipid and protein–protein interactions due to the presence of pesticide molecules or its biotransformation products in the hydrophobic domain. This would alter the membrane lipid molecular order leading to significant decrease in phosphapatidylcholine: phosphatidylethanolamine molar ratio (Lee, 2004) with increase in linear polyunsaturated fatty acids (C_{10}–C_{17}) coupled with concomitant decrease in branched acyl chain of the phospholipids. This distabilizes the membrane lipid order with profound effects on membrane proteins and ion channel functions (Cordero-Morales & Vasquez, 2018; Philips, Ursell, Niggins, & Sen, 2009). Besides, this will favor the formation of conical hexagonal geometry of the phosphatidylethanolamine in the membrane lipid microdomian with resultant changes in the biophysical and bilayer lipid composition in the microenvironment (Donato, Jurado, Antunes, & C. and Madeira, V., 1997; Diaz & Retamal, 2018; Lee, 2004; Tillman & Cascio, 2003). This view is also in accord with the suggested mechanism proposed by Habeck, Kapri-Pardes, Sharon, and Karlish (2017) that pesticides affect the lipid bilayer by incorporating the molecules or its metabolites into the hydrocarbon region or by adsorption of the molecule into both the polar and non-polar regions.

Besides, membrane lipid peroxidation by non-enzymatic processes will yield cytotoxic products that will alter the lipid bilayer physicochemical properties (Ayala, Muñoz, & Argüelles, 2014; Cazzola et al., 2011; Catalá, 2013; Cholewski, Tomczykowa, & Tomczyk, 2018) resulting in the reduction in the degree of membrane fluidity. These cytotoxic products especially the aldehydes in the form of malondialdehyde, will selectively react with the sulfydryl (-SH) groups of the enzyme to form adducts which bring about the enzyme inhibition and damage to biological molecules (Ayala et al., 2014; Bhattacharya, 2000; Cazzola et al., 2019; Zarkovic, Cipak, Jaganjac, Borovic, & Zarkovic, 2013). Studies by Habeck et al. (2017) showed that Na^+/K^+-ATPase activity depends on such lipids as phospholipids especially phosphatidylserine and cholesterol that stabilize the enzyme in the transmembrane vicinity and polyunsaturated phosphatidylcholine or phosphatidylethanolamine that activates the catalytic functions of the enzyme. They noted that the enzyme will be inhibited as cholesterol and sphingomyelin bind with β-transmembrane and α-M3, α-M5, and α-M7 domains. Also, Gad (2011) maintained that the ATPase activity in tissues will depend in the status of the membrane phosphohilid bilayer as changes in the lipid organization and structure will affect the enzyme activity. Some studies (Mahmmond & Cornelius, 2002; Tahir & Lokhandwala, 2001; Ulbricht, 2005; Waugh, 2019) showed that the inhibition of Na^+/K^+-ATPase activity could be due to activation of dopamine D-like receptors in the presence of contaminants, resulting in the phosphorylation of serine-18 α-catalytic subunit of the enzyme by protein kinase C (PKC). Furthermore, Waugh (2019) attributed the inhibition of the enzyme to the effect of the pesticide on the genes modulating its activity or to the modification of the enzymatic pathways modulating ATP and the enzyme activity. Oluah (2019) reported increased lipid peroxidation in *C. gariepinus* treated with ODZ which may suggest that the structural and functional integrity of the membranes may have been adversely compromised following increased free radical production particularly reactive oxygen species and malondialdehyde, a product of lipid peroxidation. This condition according to Zaidi, Fernandes, Bean, and Michelis (2009) is known to alter membrane lipid functionality, organization, and/or conformational status in the fish resulting in the inhibition of the Na^+/K^+-ATPase activity. This view was supported by Hazarika et al. (2001) and Ksheerasagar et al. (2011) when they observed that peroxidation of the membrane lipids will not only affect the structural and functional status of the membrane but will also affect the activities of membrane-bound enzymes like the ATPases. Besides, it is possible that ODZ or its metabolites may have direct effect on the structure of the enzyme itself leading to low enzyme activity. Cazzola et al. (2019) also attributed reduced Na^+/K^+-ATPase activity to lipid peroxidation by non-enzymatic processes. The observed significant dose and duration-dependent decrease in the Na^+/K^+-ATPase activity in this study may according to Cazzola et al. (2019) be attributed to the selective reaction of the cytotoxic products of lipid peroxidation with the thiol moiety in the enzyme. Furthermore, Parvez et al. (2006) noted that aquatic pollutants induce changes in the ATPase system through the partitioning of the enzyme complex. This, according to Nayak et al. (2019), will induce allosteric alterations that tend to reduce the enzyme activity. Studies by Tahir and Lokhandwala (2001) demonstrated that tissue Na^+/K^+-ATPase may be inhibited by the

activation of dopamine D_1-like receptors that acts as α-adrenoceptor agonist by stimulating the dopamine (DA) receptor sites. This regulatory mechanism according to Clark, Khan, and Baro (2008) and Therien and Blostein (2000) entails G-proteins that are coupled to D_1 receptors like protein kinase C and the associated pathways that promote the formation of cAMP. The mechanistic details of the role or involvement of protein kinases in the regulation of Na^+/K^+-ATPase involves the stimulation of adenyl cyclase activity by dopamine D_1 receptors to increase cAMP formation unlike D_2 -like receptors, to promote phosphorylation of Na^+/K^+-ATPase and consequent internalization or endocytosis and inactivation of the enzyme (Clark et al., 2008; Arnaldo et al., 2014). Endocytosis of the sodium pump as a means of inhibiting Na^+/K^+-ATPase other than direct phosphorylation has also been reported in *Xenopus* (Vasilets, Schmalzing, Madefessel, Hamse, & Schmalzing, 1990). This mechanism according to Therien and Blostein (2000) helps to retain the Na^+/K^+-ATPase in inactive phosphorylated state, thereby inhibiting the enzyme activity. It is our belief that ODZ may also have induced Na^+/K^+-ATPase inhibition in the tissues of *C. gariepinus* by acting on the hormone dopamine, a catecholaime. The activation of the PKC by toxicants and subsequent phosphorylation of the catalytic α-subunit of Na^+/K^+-ATPase represents a major process underpinning the dopamine-induced inhibition of Na^+/K^+-ATPase activity by endocytosis (Chibalin et al., 1998; Cinelli, Efendiev, & Pedemonte, 2008; Jung, Ryu, Ki, Woo, & Lee, 2018).

The changes in Ca^{2+}-ATPase and Na^+/K^+-ATPase activities observed in this study could predispose the fish to neuronal and cellular disorders characterized by ionic transmembrane transport impairment, disruption in the release of neurotransmitters at synaptic clefts, and ionic pump malfunction. It may also engender enhanced membrane excitability arising from prolonged depolarization of cells. The stimulation of Ca^{2+}-ATPase activity reported in this study may lead to imbalance in Ca^{2+} homeostasis that could result in the displacement of Mg^{2+} from their normal binding sites thereby facilitating renal Mg^{2+} loss. The inhibition of Na^+/K^+-ATPase activities coupled with the stimulation of Ca^{2+}-ATPase may lead to osmoregulatory perturbations in the chloride cells (Perry, 1997). Besides, the observed changes in enzyme activities in this study may trigger other physiological and biochemical events that are likely to aid in the compromise of body functions. It could also indicate the development of energy-related disturbances that may have far-reaching metabolic and physiological consequences in the fish as they act as signal transducers (Gable et al., 2014). It could also result in poor utilization of ATP to generate sufficient energy for metabolic functions, reduce Ca^{2+} transport by the Na^+/Ca^{2+} exchanger, and may probably disturb the Na^+ and K^+ pump that would likely result in unregulated Na^+ and K^+ movement in the tissues along the concentration gradient while water will flow along the osmotic gradient (Shwetha et al., 2012). Such ionic and osmotic imbalances could cause cellular swelling and eventual membrane rupture (Oruc, Uner, & Tamer, 2002).

Conclusion

In conclusion, since these enzymes are not only intimately involved in the active ion transport processes and maintenance of membrane ionic balance but are also intricately involved in other cellular functions, the observed changes in their activity following exposure to ODZ may result in impaired metabolic functions, osmoregulatory capacity, and neurodegenerative and vascular pathologies in the fish. Accordingly, the determination of the activities of these enzymes could be diagnostically used to evaluate and monitor physiological changes in the fish and to measure the impacts of herbicides on aquatic fauna. Thus, the result of this study suggests strongly that the assay of these enzymes could justifiably be used as early biosignal of aquatic pollution.

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Ethical approval

This study was carried out in compliance with the guidelines issued by the Ethical Committee of the Faculty of Biological Sciences, University of Nigeria, Nsukka that granted the permission to carry out the study.

Authors' contributions

NSO participated in the design of the study and carried out the study as part of his doctoral work, collected the data, and wrote the first manuscript. BOM participated in the design and supervision of the study and read the manuscript. CDN read and corrected the manuscript. IOA helped in the statistical analysis and read the manuscript. CO was involved in the laboratory analysis, and she read the manuscript. CIN read the manuscript and participated in laboratory work. The authors read and approved the final manuscript.

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Competing interest

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Consent for publication

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