ejpmr, 2018,5(4), 130-135

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

CHANGES IN ELECTROLYTES LEVEL OF *CLARIAS GARIEPINUS* (BURCHELL 1822) EXPOSED TO SUBLETHAL CONCENTRATIONS OF OILFIELD WASTEWATER

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Article Received on 09/01/2018 **Article Revised on 01/03/2018** **Article Accepted on 21/03/2018**

ABSTRACT

The electrolytic changes of twenty-eight adult *Clarias gariepinus* (Burchell, 1822), (mean weight 205 ± 12.89g SD; Mean length; 31.13 ± 3.82cm SD) exposed to sub-lethal concentrations (0, 10, 20, 30, 40, 50 and 60%) of an oilfield wastewater over a period of twenty eight (28) days were studied in a semi-static system. At the end of 28 days, tissue/organ and plasma samples were collected for electrolysis using standard methods. Insignificant ($p \leq 0.05$) fluctuations in electrolytes (sodium, potassium, chloride, calcium and bicarbonates ions) were observed in exposed fishes when compared to the control group. These fluctuations in electrolytes suggest the effort made by the fish to combat the stress imposed by the toxicant. The ions (electrolytes) are involved in regulation of osmotic changes in the fish and can be used to measure long term effect of *C. gariepinus* exposed to oilfield wastewater. The proper treatment of oilfield wastewater prior to discharge into the recipient water body is advocated to reduce ecotoxicological problems.

KEYWORDS: *Clarias gariepinus*, ecotoxicology, electrolytes, oilfield wastewater, organ, tissue.

INTRODUCTION

Electrolytes are charged minerals found in organisms with enormous functions.[1] These electrolytes include sodium, potassium, chloride, calcium and bicarbonates. Sodium and potassium are the major cations of the extracellular fluids while chloride and bicarbonates are the major anions of the intracellular fluids.[2,3] Grouped as divalent and monovalent ions according to functions, the divalent ions (Calcium and Magnesium) are important in neuromuscular excitability, enzymatic reactions and retention of membrane permeability.[1,4] Accordingly, the monovalent ions (Chloride, Potassium and Sodium) are key in acid to base balance (pH), osmotic pressure as well as neuromuscular excitability.[5,4] Also, inorganic phosphate is important in energy exchange as it is an important cytoplasmic buffer.[4,6,7] Electrolytes are useful in redox (electron transfer) processes and as co-factors for enzymes.[7] Sodium is essential for the activity of many enzymes and has been implicated in the transport of ATP. Sodium and potassium ATPase are located in the cell membrane and are involved in the active transport of Na^+ and K^+ across the cell membrane.[8]

Clarias gariepinus is a fish of choice in aquaculture due to its hardy nature, ease of larval production in captivity and good market price.[9,10] It is also wide bred because of their palatability, fecundity, disease resistance and high growth rate.[11] Further, *Clarias* is available at all seasons, is easy to use in the laboratory and has relative sensitivity (high level of tolerance) to petroleum products.

Anthropogenic activities have placed the environment at the receiving end of numerous contaminants.[12] Environmental contaminants are known to alter the levels of electrolytes in fish.[13,14] In recent years, the concern on the pollution of the environment by produced or formation water has been on the aquatic environments.[15,16,17] This is due to marked increase in offshore oil operations. A few studies have been carried out on the discharge of produce water or oilfield waste water from onshore operations into terrestrial environments.[18,19,20]

The potential toxicity of oilfield wastewater on exposed biota is notable, although their actual effect is not clear. The physicochemical properties of oilfield wastewater indicate that it could pose serious threat to biota.[21] The disposal of such effluents on land during onshore operations could pose a threat to exposed fish in captivity especially fish ponds located around such area. However, not much data is available on the effect of this oilfield waste water on fish and other biota. Fish live in very intimate contact with their environment.[22,23,24] These indices have been effectively employed in monitoring the responses of the fish to the stressors and evaluating its health status under such adverse conditions. This study therefore aims at evaluating the electrolyte changes of *C.*

gariepinus exposed to sublethal concentrations of oilfield wastewater.

MATERIALS AND METHODS

Test Organism

Twenty-eight adult (mean weight 205 ± 12.89g SD; mean length; 31.13 ± 3.82cm SD) of *C. gariepinus* were obtained from the African Regional Aquaculture Center (ARAC) at Aluu in Ikwerre Local government area of Rivers State. The *C. gariepinus* were put into 50 L trough containing borehole water and sealed with a net before transporting to the laboratory for analysis. The fish were then acclimated individually in covered rectangular aquaria for two weeks. The water was changed daily and the aquaria washed with a piece of foam and water. All samples were fed twice with a 35% crude protein diet at 1% biomass daily (8.00 a.m. and 5.00p.m.). Mortality during acclimation was less than one percent. All fish samples were treated in line with recommended scientific procedure.

Test toxicant (oilfield wastewater)

The test toxicant (oilfield wastewater) was collected from Ebocha oilfield in Ogba/Egbema Local Government Area of Rivers State with coordinates N05 27´ 40.45´ E006 41´ 52.14´. The effluent was collected in 50 litres plastic containers on three occasions and transported to the laboratory. These represented different ranges of the discharge at the discharge point.

Optimization Test (Preliminary Investigation)

The range or concentrations of oilfield wastewater that exhibited lethal and sub-lethal effect on the fingerlings and adult of *C. gariepinus* respectively was determined using standard procedures. Five concentrations (10%, 30%, 50%, 70% and 100%) of the oilfield wastewater were prepared by serial dilution from each effluent sample on a volume to volume (v/v) ratio. The percentage (%) concentration in each test solution was obtained by using the formula below[25]:

$$Concentration\ of\ effluent(\%) = \frac{V_E}{V_E + V_{DW}} \times 100$$

Where,
V_E = Volume of effluent
V_{DW} = Volume of dilution water

The determined volume of effluent was added to the desired quantity of dilution (borehole) water and vigorously mixed.

The water was not changed for a period of one week. However, the fish were fed twice daily as in the acclimation period. Concentrations that caused death within one week were omitted from the definitive test.[26] Since the fingerlings did not die after 96 hours of exposure the lethal test for the fingerlings was discontinued.

Sub-lethal test

Based on the results from the optimisation test, 10, 20, 30, 40, 50 and 60%v/v of oilfield wastewater were prepared as the sub-lethal concentrations and used for the study, including a control. Each treatment was set up in four replicates. A fish was then introduced into each of these concentrations contained in an aquarium and incubated for a period of 28 days at room temperature (30±2°C). Fifteen litres of each prepared concentration was used and fish was fed as in the acclimation period. The test solution was renewed weekly after washing the aquarium with a piece of foam to get fresh toxicant to maintain concentration.

Electrolyte Analysis

After 28 days, blood samples were collected from the fish (behind the anal fin) using a 23G size needle and syringe for electrolyte analysis. Then, the Fish were killed and dissected to obtain samples of the gill, liver, kidney, and muscle tissue. Using pestle and mortar, 0.5g each of the organ/tissues were macerated. To each of the samples, 5ml of deionised water was added, centrifuged at 3000 rpm for 10 minutes. The supernatants were then extracted and stored in plain bottles at -20°C and electrolytes analysed.

The electrolytes analyses were done for Sodium (Na^+), Chloride (Cl^-), Calcium (Ca^{2+}), Potassium (K^+) and Bicarbonates (HCO_3^-) according to Ochei and Kolhatkar.[4]

Calculations

Absorbance of blank — absorb of sample x conc. of standard Absorb. of blank — absorbance of standard = concentration of K (mmol/ L).

RESULTS AND DISCUSSION

Environmental pollutants have been known to alter levels of electrolytes in fish[13,14] and can be used to monitor the responses of the fish to the stressors and evaluating its health status under such adverse conditions.

In this study, significant changes ($p \leq 0.05$) in the electrolytes of *C. gariepinus* exposed to oilfield wastewater was observed between the control and some treated groups as well as among the treated fish (Table 1). Although no given trend was observed, the value of sodium in plasma ranged between 65.75±8.09 mmol/l at 30% and 142.00±7.48 mmol/l at the control; Chloride ranged between 108.00±16.17 mmol/l at 20% and 134.75±1.50 mmol/l at 40% and Calcium ranged between 6.00±0.00mmol/l at 50% and 10.50±1.73mmol/l at 30%. Similarly, Potassium ranged between 6.20±0.48mmol/l at 20% and 8.55±0.48mmol/l at 40% while bicarbonates ranged between 3.70±0.00mmol/l at 30% and 8.48±4.71mmol/l at 50% of the toxicant.

Table 1: Electrolytes in the Plasma of C. gariepinus exposed to different concentrations of an oilfield wastewater after 28 days.

Conc. of Oilfield wastewater (%)	Electrolytes(mmol/l)				
	Na^+	Cl^-	Ca^{2+}	K^+	HCO^3
0	142.00±7.48[e]	110.50±6.35[ab]	7.50±0.58[ab]	6.48±0.61[a]	6.50±3.07[a]
10	84.00±3.65[b]	122.00±13.86[abc]	9.50±2.89[bc]	6.95±0.52[a]	6.53±2.98[a]
20	131.00±4.16[e]	108.00±16.17[a]	7.50±2.89[ab]	6.20±0.48[a]	5.55±0.06[a]
30	65.75±8.09[a]	133.50±.58[c]	10.50±1.73[b]	6.78±0.51[a]	3.70±0.00[a]
40	99.50±9.98[c]	134.75±1.50[c]	8.00±1.16[ab]	8.55±0.48[c]	7.35±2.02[a]
50	118.75±2.22[d]	127.00±0.00[bc]	6.00±0.00[a]	7.43±1.25[ab]	8.48±4.71[a]
60	112.00±12.96[d]	127.50±6.35[bc]	6.50±0.58[a]	7.53±1.03[ab]	5.55±2.14[a]

∗Means with the same superscript in the column are not significantly different at $p \leq 0.05$

Electrolytes in the gills of *C. gariepinus* showed a significant difference ($p \leq 0.05$) at the determined concentrations, although they did not follow any trend (Table 2). Electrolytes in the liver of *C. gariepinus* exposed to the toxicant are as shown table 3. There was a significant difference at $p \leq 0.05$. Generally higher values were recorded for sodium, chloride, calcium and potassium at 60% of the toxicant, but at the other concentration including the control there was no trend followed. Bicarbonate was highest at 50% while at other concentrations there was no significant difference at $p \leq 0.05$. Similarly, electrolytes in kidney of *C. gariepinus* exposed to the toxicant showed a slight difference ($p \leq 0.05$) at the constituted concentrations although with no defined trend (Table 4).

Table 2: Electrolytes in the gills of *C. gariepinus* exposed to different concentrations of an oilfield wastewater after 28 days.

Conc. of Oilfield wastewater (%)	Electrolytes (mmol/l)				
	Na^+	Cl^-	Ca^{2+}	K^+	HCO^3
0	557.50±68.07[a]	125.00±16.17[a]	5.00±0.00[a]	13.75±3.20[a]	20.00±5.77[a]
10	622.50±72.74[a]	120.00±15.00[a]	15.00±5.77[b]	12.50±0.71[a]	25.00±0.00[a]
20	982.50±56.79[b]	138.13±38.42[b]	5.00±0.00[a]	14.50±0.58[a]	36.25±9.47[b]
30	502.50±12.58[a]	83.50±0.00[a]	5.00±0.00[a]	16.25±1.44[a]	45.00±5.77[b]
40	581.25±56.77[a]	96.75±15.30[a]	10.00±5.77[a]	18.75±4.33[a]	58.75±11.82[c]
50	535.00±87.37[a]	110.00±0.00[a]	5.00±0.00[a]	15.00±0.00[a]	22.50±2.89[a]
60	500.00±8.17[a]	122.50±14.43[a]	5.00±0.00[a]	17.63±5.63[a]	45.00±5.77[b]

∗Means with the same superscript in the column are not significantly different at $P \geq 0.05$.

This study demonstrated fluctuations in ions of fish exposed to concentrations of oilfield wastewater as compared with the control. Also, test groups showed significant changes in the electrolytes level in the organs studied. However, no remarkable trend was observed in most results. This may be due to disturbances in ionic concentration of the water. Studies have shown the need for strict ionic regulation (homeostasis) in aquatic animals.[27] There were significant changes ($p \leq 0.05$) in plasma electrolytes in the fish studied except bicarbonates that did not change at the various concentrations of the wastewater. Plasma electrolytes are known to fluctuate in response to various factors.[28] These disturbances in homeostasis are induced by pollutants evidenced by altered plasma ion concentration.[28] Other studies report that the concentrations of monovalent ions in plasma are commonly altered in response to pollutants.[2930] The fish in the present study showed fluctuations due to exposure to produced water (a pollutant). Sodium, potassium and calcium are strongly electropositive and not easily oxidized or reduced.[3] Thus biologically, they have a major role in neutralizing the charge on anions and in maintaining isotonic pressure in natural fluids.[31] This was observed in this study.

Table 3: Electrolytes in the Liver of C. gariepinus exposed to different concentrations of an oilfield wastewater after 28 days.

Conc. of Oilfield wastewater (%)	Electrolytes (mmol/l)				
	Na^+	Cl^-	Ca^{2+}	K^+	HCO^3
0	608.75±31.19[b]	83.50±0.00[a]	5.00±0.00[a]	15.75±0.87[c]	50.00±0.00[a]
10	567.50±39.69[b]	69.25±16.46[a]	7.50±2.89[a]	17.25±0.96[c]	40.00±0.00[a]
20	550.00±8.17[b]	96.75±15.29[a]	5.00±0.00[a]	7.00±1.83[a]	45.00±5.77[a]
30	270.00±21.60[a]	110.00±0.00[a]	11.25±6.29[a]	6.50±1.73[a]	45.00±5.77[a]
40	608.75±63.03[b]	83.50±0.00[a]	7.50±2.89[a]	13.25±2.02[b]	62.50±14.43[b]
50	558.75±26.89[b]	82.50±31.75[a]	12.50±8.66[a]	22.75±2.22[d]	45.00±5.77[a]
60	717.50±23.63[c]	166.50±31.75[b]	15.00±5.77[a]	34.00±0.58[e]	45.00±5.77[a]

∗Means with the same superscript in the column are not significantly different at $p \leq 0.05$

Table 4: Electrolytes in the Kidney of *C. gariepinus* exposed to different concentrations of an oilfield wastewater after 28 days.

Conc. Of Oilfield wastewater (%)	Electrolytes(mmol/ L) Na+	Cl -	Ca2	K+	HCO3-
0	745.00±72.34c	83.50±0.00a	5.00±0.00a	17.50±1.73b	35.00±5.77a
10	455.00±30.28a	83.50±0.00a	5.00±0.00a	17.00±2.31b	42.50±9.57a
20	650.00±47.61b	83.50±0.00a	12.50±2.89b	16.25±1.44b	67.50±8.66b
30	500.00±23.81a	83.50±0.00a	7.50±2.89a	18.25±0.87b	45.00±5.77a
40	477.50±22.17a	69.25±16.45a	5.00±0.00a	23.25±0.87c	45.00±5.77a
50	446.25±40.29a	83.50±0.00a	7.50±2.89a	16.75±0.87b	40.00±0.00a
60	645.00±42.03b	69.25±16.46a	5.00±0.00a	8.63±1.32a	40.00±11.55a

∗Means with the same superscript in the column are not significantly different at P≤0.05.

In muscle of *C. gariepinus*, significant difference ($p \leq 0.05$) was observed in the value of electrolytes at the constituted concentrations (Table 5). Sodium, chloride and bicarbonate recorded higher values of 792.50±17.08mmol/l, 165.00±0.00mmol/l and 45.00±5.77 mmol/l respectively at 60% while calcium and potassium was the reverse as higher values of 20.00±8.17mmol/l and 46.00±2.94 mmol/l were recorded in the control (0%). The results did not follow any particular trend.

Table 5: Electrolytes in the Muscle of C. gariepinus exposed to different concentrations of an oilfield wastewater after 28 days.

Conc. of Oilfield wastewater (%)	Electrolytes(mmol/l) Na+	Cl -	Ca2+	K+	HCO3-
0	515.00±69.52c	110.00±0.00c	20.00±8.17b	46.00±2.94c	25.00±5.77a
10	510.00±75.28c	96.75±15.30b	5.00±0.00a	27.75±2.02a	25.00±5.77a
20	332.50±8.66b	110.00±0.00c	5.00±0.00a	36.00±0.58b	25.00±5.77a
30	305.00±5.77ab	83.50±0.00a	5.00±0.00a	26.75±0.96a	35.00±17.32a
40	257.50±9.57a	83.50±0.00a	5.00±0.00a	33.50±2.94b	40.00±11.55a
50	597.50±17.08d	83.50±0.00a	16.25±6.29b	29.63±1.44a	45.00±5.77a
60	792.50±17.08e	165.00±0.00b	8.75±4.79a	27.13±1.89a	45.00±5.77a

∗Means with the same superscript in the column are not significantly different at $p \leq 0.05$.

Changes in the electrolytes level had no trend in all the parts studied. This could be based on the methods of analysis used and various factors both exogenous and endogenous.[32] It may be necessary to consider these factors in future studies. Fish in disturbed osmotic environment behaviorally adjust their electrolyte.[32] This further supports the lack of trend in the results of the present study. The gills play important role in the behavioral ionic regulation of fish. The results on the electrolyte levels of the gills of the test fish show effort to neutralize the effect of pollutants as in other studies.[9,10] This is also true for the kidneys. This further demonstrates the hardy nature of *C. gariepinus*.

The lack of trend in the electrolytes analysed in these organs may be due to stress induced degeneration of these organs.[33,32] These studies report that exposure to toxicants could lead to degeneration. Kidney degeneration has been implicated for reabsorbtion.[33,34,29] This may explain uneven trends in calcium, phosphates and other electrolytes.

Similarly, fluctuations were observed in the chloride and bicarbonates levels in the tissues of exposed fishes when compared to the control group. Gabriel *et al.*[14] noted that levels of electrolytes in organs and tissue of *Heterobranchus bidorsalis* exposed to concentrations of cypermethrin were either higher or lower than the control value. This is a reflection of changes in the fluxes of the electrolytes as a result of cypermethrin toxicosis. The presence of oilfield wastewater may be responsible for the fluctuations observed in this study.

Further, the fluctuations in electrolytes analysed could be linked to heart stress. Reduction of Na^+ ion level may affect the heart functions and cause neurotoxic damage to the central nervous system (CNS) of fish.[35] According to Cox[36] cypermethrin alters nerve impulse travels along nerves of vertebrates and other animals. The nerves become momentarily permeable to sodium atoms, thus allowing it to flow into nerves. It also delays the closing of the gate that allows the sodium flow.[37] This eventually results in multiple nerve impulses instead of the usual single. In turn, these impulses cause the nerve to release the neurotransmitter, acetylcholine and then stimulate other nerves.[14] Multiple impulses may result when fish are exposed to toxicants.

Further, the lack in trend observed in this study could indicate osmoregulation in the fish tested. Electrolyte concentrations have been used as an indicator of fishes' ability to osmo-regulate.[38] This is often compromised with stress, disease or gill lesions that increase gill permeability to ions or lateral line imbalance and hormonal disorder.[39] Electrolytes have been shown to play a central role in gaseous exchange and intercompartmental water balance.[4] Hence elevated or low levels may result in hyper or hypo functions of tissues or organs of fish.[40,14]

CONCLUSION

The current study observed fluctuations in electrolytes in the fish exposed to sublethal concentrations of oilfield wastewater. This indicates the effort made by the fish to combat the stress imposed by the toxicant. The ions (electrolytes) involved in regulation of osmotic changes in the fish can be used to measure long term effect of *C. gariepinus* exposed to oilfield wastewater. The proper treatment of oilfield wastewater prior to discharge into water body is advocated to reduce ecotoxicological problems.

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