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Modulations in Carbohydrate Metabolic Pathways and Hydrocortisone Secretion in *Clarias gariepinus* Induced with Nonylphenol Ethoxylates

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Abstract

The sublethal impacts of nonylphenol ethoxylates (NPE) on glucose metabolism and endocrine disruption in *Clarias gariepinus* were examined. Exposure to sublethal concentrations of NPE (0.50, 0.75, 1.00, and 1.25 mg/L) induced alterations in glucose levels and corticosteroid secretion. Blood samples for glucose were obtained from the caudal vein and analysed by the glucose oxidase method, while cortisol levels were assessed using enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer's protocol. Initially, glucose levels rose after two days, followed by a marked decrease as exposure continued, with significant alterations noted on day 30 ($p < 0.05$), except for the 0.05 mg/L treatment ($p < 0.05$). The initial increase in glucose is associated with glucose serving as the principal energy source and a marker of stress response. Cortisol levels rose with time in all treatment groups, exhibiting statistically significant differences ($p < 0.05$; $p < 0.01$). The findings indicate that NPE functions as an endocrine disruptor, elevating glucose levels and altering hormonal balances. This suggests that NPE exposure could have comparable impacts on human health, possibly resulting in lethal outcomes. Given that the majority of NPE exposure arises from human activities, it is imperative for authorities to enforce more stringent measures to mitigate the associated hazards.

Key words: *Nonylphenol ethoxylates, Clarias gariepinus, Hydrocortisone, Glucose, Plasma*

1. Introduction

Recent developments in Nigeria and other emerging countries have highlighted the importance of water resource contamination as a critical issue. One of the primary reasons for this is the pollution of the aquatic environment by a large number of harmful and bioaccumulative substances (Esenowo and Ugwunba, 2010). Only a few chemicals have been assessed for safety in an ecological setting in Nigeria, even though they have significant environmental consequences. In recent times, the Nigerian Federal Government has placed significant emphasis on the imperative of sufficient environmental protection in all technological and socioeconomic advancements. This is evident via the implementation of regulations that mandate industrial

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operators to effectively manage the disposal of chemicals within natural ecosystems.in strict accordance with international standards (DPR, 2002).

Alkylphenols, which include nonylphenols (NP), are a subclass of alkylphenols. Sludge, river sediment, as well as other environments, accumulate and retain the surfactant. Solubilizers, detergents, emulsifiers, and antioxidants are some of the products they are used to make (Soares *et al.*, 2008). As well as serving as precursors to other commercially important nonionic surfactants, such as alkylphenol ethoxylates and nonionic polymers (NP ethoxylates), these chemicals can be found in a wide range of everyday household and personal care items. As a result of NP's widespread presence in the environment and its possible roles as an endocrine disruptor and xenoestrogen, it has gained attention (MPCA, 2009). NP is xenoestrogen-like endocrine disruptor because they bind to estrogen receptors and compete with natural estrogens for binding (Lee *et al.*, 2004). By mimicking the natural hormone 17-estradiol, NP has been shown to compete with the endogenous hormone for binding to estrogen receptors ER and ER (Kosswig *et al.*, 2009). As a result, nonylphenol ethoxylates are frequently found in municipal and industrial wastewater (Lee, 2004).

Detergents, cleaners, agricultural and indoor insecticides, food packaging, and cosmetics all contain NP, putting consumers at risk. Women and children may be concerned about these products. NPEs and NP can be found in floor and carpet cleaners, exposing little toddlers to them. According to the European Union Risk Assessment, NP production, and consumption are conducted in closed systems. Biomonitoring studies from breast milk (Ademollo *et al.*, 2008), umbilical cord blood (Chen *et al.*, 2004), and urine have confirmed general population exposure to NP (either directly or as a metabolite of NPE) (Calafet *et al.*, 2005). The highest amount of NP detected in breast milk was 56.3 g/L, resulting in an estimated daily dosage of 3.9 g/kg for a child (Ademollo *et al.*, 2008).

Workers may be exposed to hazardous materials during sampling, maintenance, and product filling of drums and tankers (EC, 2005). For both the manufacture and usage of NP, occupational exposure was calculated to be 0.9 mg/m3 (8-hour TWA) (EC, 2005). Use of NP in the production of NPEs, or processing or use of NPEs in industrial and institutional cleaning products, emulsion polymerizations, textiles, pulping and paper-based products, electrical and electronic products, metal extraction-processing, or lacquers-and-varnish manufacturing, were all discovered as potential NPE applications

Several of these sources have provided estimates of human exposure. According to Guenther et al. (2002), a 70 kg person is exposed to 0.01g kg/day of diet; hair dye is 0.01%; food containers are 0.02%, and indoor pesticides are 0.35 grams per kilogram of body weight daily (EU, 2002). NP exposure from environmental sources is estimated at 5g/kg/day, with 70% to 80% of this coming from fish and shellfish diet, according to a European Union assessment (EU, 2002). The consumption of seafood and NP levels in breast milk were found to be linked in a biomonitoring investigation (Ademollo *et al.*, 2008). In addition, hard surface cleaners and their residue could pose a significant risk to health and safety. Living close to a textile plant where NPE and NP were utilized resulted in the highest estimate of exposure (4.4 mg/kg/day) (EU, 2002). The 95th percentile exposure level for water consumption is 2.544 litres per day, with the average adult consuming about 0.926 litres of water per day (EPA, 2009). An estimated 0.01g/kg/day of exposure to alkylphenols in drinking water can be calculated by combining the measured value of 1g/L and the average daily consumption estimate of 0.926 L. The EPA concludes that drinking water should not be the primary source of exposure, a view that is shared by other sources of information (Soares *et al.*, 2008). NP and NPEs may pose a risk to children's health. Laboratory animals exposed to NP over numerous generations had their oestrous cycles, vaginal opening times, ovarian weights, and sperm/spermatid counts somewhat altered (EU, 2002). Exposure to NP across several generations has been shown to have a small but statistically significant effect on the length, time, and ovarian weight of the oestrous cycle, the timing of vaginal opening, and the sperm and spermatid count in experimental animals (Chapin *et al.*, 1999). An investigation into the NP's mode of action has not been done, which is compatible with the findings of this study. Because children eat, drink, and breathe more than adults, they may be exposed to more NPs and NPEs than adults because they ingest a bigger volume per pound of food, water, and air. Despite the lack of biomonitoring data, a recent Italian study indicated that human breast milk can expose children to as much as 3.9 g/kg/day of BPA (Ademollo, 2008).

The fish is a typical air-breathing, has a helmet-shaped head and a scaleless, elongated body with long dorsal and anal fins. The dorsal surface of the fish varies in colour from dark brown to light brown and frequently undergoes moulting to reveal shades of olive and grey; the underside is delicate cream to white (Skelton, 2001). It has the potential to attain substantial dimensions, peaking at 170 cm in length and 60 kg in weight (IGFA, 2001). It thrives in highly turbid and oxygen-depleted water, and is often the sole or last fish species discovered in pools that have been drained by rivers (Van der Waal, 1998). It can survive for long periods out of the water thanks to a particular suprabran organ that allows it to tolerate very low oxygen concentrations (Hecht *et al.*, 1988). This organ is a sizable paired chamber with branches situated above the gill arches. Its unique anatomical design facilitates air-breathing and enables it to traverse land even in the absence of arid conditions (Mama and Maloiy, 1986). Acceptable parameters include salinities ranging from 0 to 10 per cent, temperatures between 8 and 35 degrees Celsius, and a broad pH range (Safried and Bruton, 1984). The rapid development of *C. gariepinus* is observed at a temperature of 30°C. The fish's ability to endure such high temperatures enables it to persist in environments containing moist sediment or air-water interface deposits (Van der Waal, 1998).

The human and ecological disturbance experienced in industrial settlements as a result of improper chemical disposal, such as detergent effluent, necessitates close monitoring of the state of the environment.

2. Materials and methods

2.1. Pre-analytical stage

This investigation was conducted under all applicable laws and guidelines. A greenhouse that resembled the fish's natural habitat was built, and mini ponds measuring 270 1/2 x 24 1/4 x 29 12 inches were constructed with clayey loam soil, within the greenhouse. Self-bred *C. gariepinus* of equal length and weight were used in this study, and they were monitored from the egg to the stage of maturity that was desired. The fish were fed on protein- and vitamin-rich fodder twice daily. They underwent routine exams to assess their well-being, and level of maturity. The brood fish pond's water is periodically drained and replaced with fresh water to maintain water quality. Before being toxicologically tested, the fish produced by these setups were allowed to grow for 20 weeks.

2.2. Experimentation

The fish was carefully brought to the lab and acclimated for two weeks. After 14 days of acclimatization, the fish were transferred to 10-litre plastic tubs, with ten fish per tub. The fish were exposed to the field-reported Nonylphenol Ethoxylates concentration ranges for 30 days (0.50, 0.75, 1.00, and 1.25 mg/L, including the control). Fish were fed twice daily at 3% of body weight to the control and experimental groups throughout the experiment.

The plastic tubs were kept as spotless as possible, and the water and poisons were completely replaced each day. Throughout the investigation, daily assessments of the water's physicochemical properties were made. A fish was removed from each plastic tub after each experimental period—the second, ninth, sixteenth, and thirtieth —and its heart was punctured to obtain blood samples, which were kept in labelled test bottles, until analysis.

2.3. Determination of glucose level

Glucose levels can be measured in whole blood, plasma, or serum samples. The concentration will be lower if whole blood is used instead of plasma or serum. This is owing to the cellular fraction's higher water content.

The Beckton Dickinson blood sugar Assay Kit was used to measure blood sugar levels (Becton Dickinson India Pvt. Ltd., Bangalore, India). The serum (10 μl) was mixed with 90 μl of reagent, which consists of glucose oxidase–peroxidase. After 10 minutes, the Optical Density (OD) of the coloured complex was measured in an autoanalyzer at 620 nm.

2.4. Cortisol determination

Cortisol levels were assessed using enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer's protocol. The cortisol levels in duplicate plasma samples were measured using 96-well plates. Using a standard curve that was run on each plate, the marker concentration was calculated and the sample volume and dilution factor were taken into account (plasma). The inter-assay variability was 4.32 per cent, and the lower limit of detection was 52.4 pg/mL.

3. Statistical analysis

Minitab Statistical Computing System, SAS (SAS institute Inc., 1985), SPSS version 16.0 (Chicago IL, USA), Microsoft Excel 2010 (Roselle, IL, USA), and SPSS, version 10 (IBM Corp., 2016) applications were used to analyze data. The least significant difference (LSD) was calculated using post hoc testing for treatment and control group comparisons at a probability threshold of 0.05% and 0.01%, respectively.

4. Results

In the surfactant-induced fish, the sugar levels surfed after two days in all the treatments and were concentration-dependent (Figure 1). On day 2, the sugar level varies significantly between the control and the treatment ($p < 0.05$). Similarly, the sugar level was highly significant between the control and various treatments (0.75, 1.00, and 1.25) mg/L. on day 30 ($p < 0.05$), with the exception of 0.05 mg/L ($p > 0.05$).

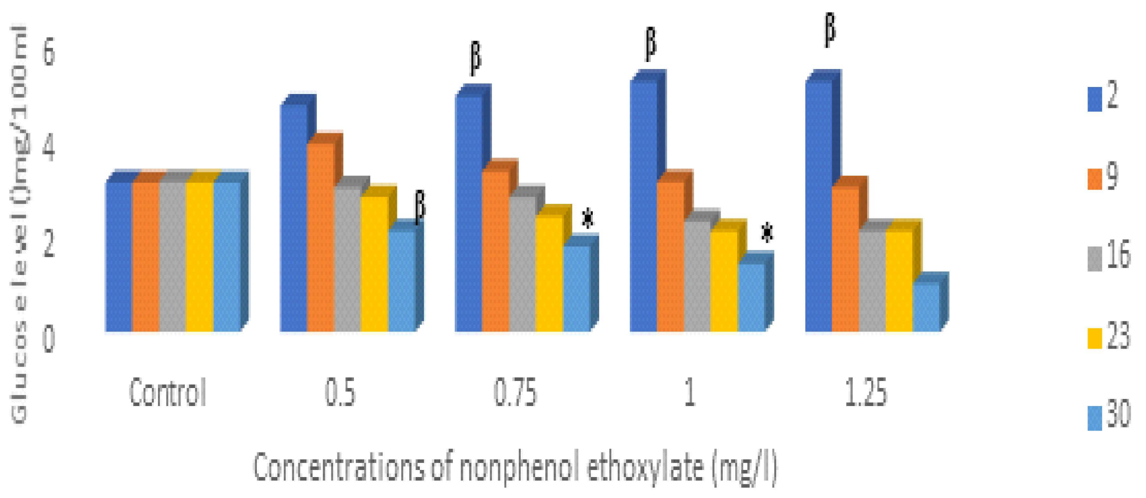

Figure 1: Variations in the glucose level in *C. gariepinus* exposed to different concentrations of nonphenol ethoxylate

Note: Data presented as mean ± SE. The symbol above chart indicates significant differences between the control and the treatments $^{\beta}(p \leq 0.05)$, $^{*}(p \leq 0.01)$.

The endocrine response in *C.gariepinus* exposed to various concentrations of nonphenol ethoxylate showed slight variation in cortisol concentration in the control with time but not significant ($p > 0.05$). In the treatments irrespective of the concentrations, the cortisol level increases with time and varies significantly ($p < 0.05$; $p < 0.01$) (Table 1).

Table 1: Responses of hydrocortisone (μg/dL) in the plasma of *C. gariepinus* exposed to sublethal concentrations (mg/l) of nonylphenol ethoxylate

Days	Control	0.50	0.75	1.00	1.25
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
2	4.21 ± 0.020^{a}	5.30 ± 0.10^{a}	5.70 ± 0.12^{a}	5.90 ± 0.32^{a}	6.00 ± 0.27^{a}
9	4.23 ± 0.013^{a}	8.30 ± 0.18^{b}	8.53 ± 0.10^{b}	10.20 ± 0.18^{b}	11.80 ± 0.10^{b}
16	4.27 ± 0.038^{a}	0.10 ± 0.25^{b}	11.50 ± 0.30^{c}	14.10 ± 0.10^{c}	15.30 ± 0.22^{c}
23	4.21 ± 0.041^{a}	11.80 ± 0.10^{b}	14.30 ± 0.13^{c}	19.30 ± 0.15^{c}	19.20 ± 0.13^{c}
30	4.21 ± 0.010^{a}	13.90 ± 0.11^{b}	15.80 ± 0.21^{c}	21.90 ± 0.22^{c}	24.10 ± 0.30^{c}

Note: ^{a} not significant ($p < 0.05$), ^{b} significant ($p < 0.05$), ^{c}highly significant ($p < 0.01$)

5. Discussion

After 48 hours of treatments, the glucose level in the fish exposed to nonylphenol ethoxylates increases with the increase in the concentrations of the surfactant. The significant ($p > 0.05$) elevation of blood glucose levels in the blood of exposed fish may be due to the mobilization of glycogen in glucose. It is a well-established fact that fish secrete large quantities of glucocorticoids and catecholamines from adrenal tissue under stress conditions. These hormones are well known to produce hyperglycemia in animals. The hyperglycemic condition registered in the fish exposed to NPE may be ascribed to increased secretion of the hormones mentioned above which induce glycolysis in the liver and muscles of surfactant-exposed fish. The glucose level decreases abruptly with days of exposure and the concentrations of the surfactant. The initial increase can be attributed to the fact that glucose is the primary source of energy and its increased plasma level is a secondary indicator of stress response in animals. Increased blood glucose levels have also been reported in *C. carpio* and in *Oncorhynchus mykiss* after exposure to sub-lethal concentrations of diazinon (Ahmad, 2011; Banaee *et al.*, 2011). It has been observed that continuous exposure to chemicals can lead to a decrease in glucose content, as shown on the 30th day. It has been noticed that serum glucose levels initially increase in the fish, and subsequently decrease until reaching a depleted level. This phenomenon may be attributed to the depletion of energy stores in response to the stress induced by the excessive buildup of the surfactant (David *et al.*, 2010). The reduced glucose levels observed in fishes with chronic exposure may potentially be attributed to impaired gluconeogenesis resulting from the presence of the surfactant (Zutshi *et al.*, 2010).

Extensive research has been conducted on the utilisation of water-based techniques for collecting hormones, specifically cortisol, in small viviparous fish species like Xiphophorus. These studies have established a strong correlation between the collected hormone levels and cortisol levels in the bloodstream, thus validating the efficacy of this method as a reliable measure of primary stress responses in teleost fish (Gabor and Contreras, 2012; Scott *et al*., 2008). In this investigation cortisol secretion increases with NPE concentrations and exposure duration. Glucocorticoid stress hormones, such as cortisol, are rapidly secreted in response to several environmental stressors, including temperature stress, confinement, exposure to hazardous substances, and salt (Barton, 2002). These hormones have been demonstrated to exert significant effects on numerous physiological processes. The regulation of cortisol production is mediated by the hypothalamus-pituitary-interrenal (HPI) axis, and the secretion of cortisol into the bloodstream is contingent upon the synthesis of cortisol inside the interrenal tissue (Mommsen *et al.*, 1999; Faught *et al*., 2012).

6. Conclusion

Based on these findings, it can be concluded that NPE disrupts the endocrine system, causes an increase in glucose levels in aquatic organisms, and significantly affects the hormonal profiles and specific growth rate of *C.gariepinus*. This study highlights the potential for NPE toxicity to induce comparable consequences for human health and, in extreme cases, result in significant loss of life. Human activities constitute the primary sources of NPE exposure; therefore, relevant authorities must enforce stringent mitigation measures to manage this risk.

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