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# Haematological and Biochemical Responses of *Heterobranchus bidorsalis* to Imidacloprid

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## ABSTRACT

*The aim of the study was to unveil the effect of imidacloprid (one of the most prevalent insecticide in the Niger Delta, Nigeria) on some blood and enzyme parameters in heterobranchus bidorsalis. Thirty five (35) adult heterobranchus bidorsalis (mean length 22.43 ±2.42 cm, mean weight, 166.70±0.33g) were acclimatized to Laboratory condition for seven days and then exposed to varying sub-lethal concentrations of the toxicant (0.28, 0.42 and 0.56 mgl-1 in a semi static bioassay for 14 days. Red blood cell (RBC), white blood cell (WBC), platelets (plat), haemoglobin (Hb), lymphocytes (Lymp) and neurophils (Neut) were determined in the blood while ALP (alkaline phosphatase) and ALP (acid phosphatase) were determined in the kidney. Across 0.00 – 0.56mg/l of the toxicants, the result revealed that white blood cells were 2.45 – 31.55 103mm-3, platelets were 409.00 – 941.50 x10-5, red blood cells were 6.40 – 7.60 106mm-3, haemoglobin were 6.55 – 11.85 gl-1, lymphocytes were 93.50 – 96.50% and neutrophil were 3.50 – 6.50%. There was significant difference ($P<0.05$) based on concentration for each of the parameters except for lymphocytes and red blood cells. White blood cell and Haemoglobin values were in a dose dependent pattern, while platelets and neutrophils values fluctuate within the experimental group. Furthermore the level of alkaline and phosphatase was significantly dependent on concentration with a value of 135.20 µ/L at 0.00 mg/l and 75.50 µ/L at 0.56 mg/l and 103.00 µ/L at 0.00 mg/l and 33.00 µ/L at 0.56 mg/l respectively. Alkaline and phosphatase values were significant in a dose dependent pattern. Both values retrogressed as the concentration of toxicant increases. Imidacloprid could be toxic at high concentration; hence, it should be used with caution. Further studies are required to evaluate the potential environmental risk of imidacloprid.*

**Keywords:** *Alkaline and acid phosphatase, Blood cells, Fisheries, Imidacloprid, Toxicant;*

## INTRODUCTION

Pesticide and other xenobiotics are threat to aquatic organisms worldwide (Ogamba et al., 2015). Aquatic ecosystem such as lakes, ponds, rivers, and oceans, tend to be the ultimate sink of pollutants such as pesticides (Kumor et al., 2009; Seiyaboh and Izah, 2017; Ojesanmi et al., 2017; Inyang et al., 2016a-f, 2017a-d). Pollution of aquatic ecosystem by natural and anthropogenic activities is a major challenge affecting its sustainability (Ubong et al, 2015; Ogundiran et al., 2010; Seiyaboh and Izah, 2017; Ben-Eledo et al., 2017a, b).

Contamination of pesticides in the aquatic ecosystemcan pose a serious threat to aquatic organisms like fishes, which are exposed to large of these xenobiotics and other anthropogenic releases (Kumar et al., 2009; Inyang et al., 2016a-f). Among the aquatic organisms, fishes play an important role in aquatic toxicology studies. This is probably due to their ability to response to biochemical stress in their habitats including bioaccumulation of toxicants. In the aquatic environment, fishes is one of the non-target organisms that could be affected by pesticides (Ojesanmi et al., 2017)

Imidacloprid is one of the major representatives of the new generation of neonicotinoid insecticides. It is a nicotine derived compound (neonicotinoid) with large potential distribution due to its agonistic action on insects over vertebrates (Tomizawa and Casida, 2011). Inidaclopid belongs to chloronicotinyl nitroguaidine chemical family (Bhardwaj et al., 2010). Once imidacloprid is introduced into the environment usually from spraying on crops or in wide urban or residential areas, it may cause problems to aquatic organisms including fishes. Studies clearly warned of the genotoxic potential of herbicides (Bokan et al., 2013) in wide range of organisms including fish.

Biochemical and physiology alterations by pesticides in fishes may lead to disruption in the normal physiological and metabolism of the organism, which may ultimately lead to fish kills (Adedeji et al., 2009). Xenobiotics are known to destroy tissues and also cause histopathological degradations as the fish show haematological responses to toxicants especially in their gills, livers, heart, kidneys and epidermis (Ubong et al., 2015; Ogundiran et al., 2010). Pesticides cause decrease conditions, behavioral abnormalities, physiological malfunction, histological, haematological and biochemical changes, cancer and gene mutation in fishes especially in juvenile (Napt, 2013; Inyang et al., 2017a-d, 2016a-f).

*Heterobranchus bidorsalis*, (a common Niger Delta wet land fish) is an air breathing catfish widely found in the northern half of African continent between Senegal and Ethiopia, as well as the Nile, it is indigenous to many rivers as Niger and Benue in Nigeria. It is widely cultured in the Niger Delta region and its adaptability in the Niger Delta ecosystem is excellent hence it is widely used as a probe organism for eco toxicological studies. Furthermore, haematological and biochemical profiles of blood can provide important information about the internal environment of the organism (Manupust, 2001). Hence, this present study was aimed at assessing the effects of different concentration of imidacloprid on haematological and biochemical parameters of *Heterobranchus bidorsalis.*

## MATERIAL AND METHODS

### (Experimental Stock)

Thirty-five (35) adult *heterobranchus bidorsalis* with mean weight, 166.70±0.33g and mean length, 22.43±2.42cm used for the study were obtained from a private fish farm at Yenagoa, Bayelsa State, Nigeria. They were transported to wet laboratory of the Department of Biological Sciences, Niger Delta University, Bayelsa State, where the assays were conducted from January to March 2017. The fishes were allowed to acclimatized individually in a rectangular aquaria for seven days during which they were fed once a day (9.00 -11.00hr) with 35% crude protein diet at 1% biomass.

### General Bioassay Techniques

Sublethal concentrations of inidacloprid (2.5EC) for the assay (0.28, 0.42. 0.56 $mgl^{-1}$) were determined based on the range finding test (Inyang et al., 2010). These were prepared by transferring 0.33. 0.55 and 0.67 mls, respectively of the original concentration of the toxicant and making it up to 25L with borehole water in the test aquaria. Another 25L of the diluents (borehole water) was used as control. The fishes were individually introduced into each aquarium and exposure period lasted for 2 week in a 48 hours renewed media. The physiochemical properties of the water used for the bioassay weredetermined using standard methods as described by APHA(1998) and the results were Temperature (26.09 – 26.04$^{o}$C), pH (6.15 – 6.19), alkalinity (13.35-15.19$mgl^{-1}$), conductivity (114.97 - 118$\mu s$/cm) and turbidity (0.49 – 0.53NTU).

After 14 days exposure period, blood samples for haematological and biochemical analysis were obtained from the probe organisms (behind the anal fin) with 23G size needle and syringe. The probe organisms were not fed prior to blood collection. Samples were preserved in EDTA. Fish were sacrificed and dissected for the collection of the kidney (for biochemical analysis). 0.5g of the kidney was macerated (grounded) with pestle and morter. Physiological saline was used for preservation and stabilization. Samples were centrifuges at the rate of 3000rpm for 10 minutes (Ogamba et al., 2015). The supernatants were then removed and stored in plain bottles at -20$^{0}$C for analysis (Ogamba et al., 2015).The alkaline phosphatase was assayed using the method previously described by Hafkenschied and Kohler (1986). The samples were analyzed at Federal Medical Centre Yenagoa, Bayelsa state.

### Statistical Analysis

The data were subjected to analysis of variance (ANOVA). Where differences exist, Duncan multiple range test (DMRT) were used to test for pairwise significant differences ($P<0.05$) between treatments (Wahua, 2002).

## RESULTS AND DISCUSSION

The full blood counts of *Heterobranchus bidorsalis* exposed to chronic levels of imidacloprid for 14 days are presented in Table 1. Red blood cell ranged from 6.40 – 7.60 $10^{6}mm^{-3}$ for 0.00 – 0.56mg/l of the toxicants, being not significantly different ($P>0.05$) except for slight apparent fluctuations. Across 0.00 – 0.56mg/l of the toxicants, the white blood cells were 2.45 – 31.55 $10^{3}mm^{-3}$, platelets were 409.00 – 941.50 $x10^{-5}$, haemoglobin were 6.55 – 11.85 $gl^{-1}$, lymphocytes were 93.50 – 96.50% and neutrophil were 3.50 – 6.50%. There was significant difference ($P<0.05$) based on

concentration for each of the parameters except for lymphocytes and red blood cells. No clear trends in values of RBC were found in the plasma of *heterobranchus bidorsalis*, however a slight apparent increase in values at 0.28 $mgl^{-1}$ and 0.42$mgl^{-1}$ were observed. White blood cells values within the experimental group were concentration dependent (values increased as the concentration of the toxicant increases). Platelet values followed the same trend as the WBC, albeit, the highest value obtained within the experimental group was low compared to the control value. Haemoglobin values fluctuate within the experimental group, and the highest value was recorded at 0.28$mgl^{-1}$ (the lowest concentration), while the lowest value was obtained at 0.56$mgl^{-1}$ (the highest concentration). The trend exhibited here showed a concentration dependent pattern (Table1). Neutrophil values were concentration dependent albeit, at 0.28 and 0.42$mgl^{-1}$, values were lower compared to the control.

Haematological parameters observed in this study indicate a variety of aberrations as a result of effect of the toxicant even at a very low concentration of 0.28$mgl^{-1}$. A significant reduction of some of the parameters implies that they are either destroyed or blood production reduced. This could result in anaemia and leucopenia as reported by Dixon and Dick (1985), Inyang (2008).

Platelets (thrombocytes) are nucleated cells which are responsible for blood clotting in fish. Retrogression in values observed in this study may signify the effect of thrombocyte production. Generally, toxic substances in the bone marrow, spleen, kidney and other haematopoitic organs may lead to pancytopenia.

White blood cell values increased slightly at the highest concentration (0.56$mgl^{-1}$) while the lowest concentration (0.28$mgl^{-1}$) recorded a significant decrease. This decrease is contrary to the believe that since leukocytes functions in organisms against foreign bodies, aided by phagocytosis and antibodies production value will increase as a result of lethal effect of the toxicant (Inyang, 2008). The exposure of fish to imidacloprid may have caused a depression in the established total immune response against a variety of antigenic substances (Inyang et al., 2014). A significant rise at the highest concentration may signify a recovery process in haematopoitic organs hence more production of leucocytes to fight against imidacloprid insults in the tissues of the probe organism.

Haemoglobin values fluctuate down the experimental group. A slight rise in values was recorded at the first concentration and subsequently, a decrease in values at the last concentration (0.56$mgl^{-1}$). Blood cells are packed with haemoglobin, the oxygen carrying protein pigment which gives blood its red colour (Taylor et al., 2005). Haemoglobin combines reversibly with oxygen to form oxyhaemaglobin in areas of high oxygen concentration (Taylor et al., 2005). The reduction of haemaglobin values may be due to poor oxygen transportation in the fish tissues.

**Table1.** *Blood cells of Heterobranchus bidorsalis exposed to chronic levels of imidacloprid for 14 days.*

| Conc. Of Imidacloprid $Mgl^{-1}$ | RBC ($10^6mm^{-3}$) | WBC ($10^3mm^{-3}$) | Plat ($X10^{-5}$) | HB ($gl^{-1}$) | Lymp. (%) | Neut. (%) |
|---|---|---|---|---|---|---|
| 0.00 | $6.50\pm0.02^a$ | $27.50\pm0.54^{ab}$ | $941.50\pm7.30^a$ | $9.30\pm0.00^b$ | $95.50\pm3.10^a$ | $5.50\pm.00^a$ |
| 0.28 | $7.60\pm0.01^a$ | $20.45\pm0.32^c$ | $409.00\pm10.10^c$ | $11.85\pm0.02^a$ | $95.30\pm3.00^a$ | $3.50\pm0.01^b$ |
| 0.42 | $7.25\pm0.21^a$ | $27.50\pm1.20^{ab}$ | $634.00\pm10.90^b$ | $10.00\pm0.01^a$ | $96.50\pm2.80^a$ | $4.50\pm0.01^b$ |
| 0.56 | $6.40\pm0.01^a$ | $31.55\pm2.10^a$ | $834.00\pm6.50^{ab}$ | $6.55\pm0.22^c$ | $93.50\pm1.90^a$ | $6.50\pm0.00^a$ |

*Means within column with different superscripts are significantly different (P<0.05)*

Lymphocytes and neutrophils form part of the granulocytes that make up leucocytes in animals. Neutrophils are more active phagocytes. In this study, a slight reduction at 0.56$mgl^{-1}$ (neutrophils) and 0.42$mgl^{-1}$ (Lymphocytes) may be akin to carbon tetrachloride and benzene which have been implicated in eliciting excess glucocortinoid and body depression, lymphatic involution and disturbance the migration of phagocytic leucocytes and obvious alteration of the total white blood cell counts (Inyang, 2008).

A concentration dependent trend was recorded in alkaline phosphatase and acid phosphatase in the kidney. All the values obtained were statistically significant (Table 2) with concentration of 135.20 $\mu/L$ at 0.00 mg/l and 75.50 $\mu/L$ at 0.56 mg/l (alkaline Phosphatase) and 103.00 $\mu/L$ at 0.00 mg/l and 33.00 $\mu/L$ at 0.56 mg/l (acid phosphatase).

The phosphatase values decreased down the experimental group as the concentration of the

xenobiotics increases. The lowest values were recorded at the highest concentration of the toxicant ($0.56mgl^{-1}$).

**Table2.** *Alkaline Phosphatase and acid phosphatase in the kidney of Heterobranchus bidorsalis exposed to chronic levels of imidacloprid for 14 days.*

| Conc. of imidacloprid $Mgl^{-1}$ | ALP $(\mu/L)$ | ACP $(\mu/L)$ |
|---|---|---|
| 0.00 | $135.20\pm4.00^{a}$ | $103.00\pm2.50^{a}$ |
| 0.28 | $111.50\pm2.52^{b}$ | $87.50\pm2.12^{b}$ |
| 0.42 | $88.00\pm0.92^{a}$ | $49.00\pm0.10^{a}$ |
| 0.56 | $75.50\pm1.10^{d}$ | $33.00\pm0.03^{d}$ |

*Means within column with different superscripts are significantly different (P<0.05) ALP (Alkaline Phosphatase), ACP (acid phosphatase).*

Alkaline and acid phosphatase test are part of standard laboratory tests to detect health aberrations in organisms (Ayalogu et al., 2001; Giboney, 2005). Alterations in these enzyme activity of fish resulting from toxicant or contaminant have effects in various organs of fish have been reported in literatures (Inyang, 2008, Inyang and Ollor, 2015; Inyang et al., 2016e). Variation in biochemical activities in fish are aimed at maintaining equilibrium in the presence of these toxicants that disrupt physiological and biochemical processes (Wedemeyer and Mcleay, 1981). Activities of these enzymes decreased in the kidney as the concentration of the toxicant increases (in a dose dependent pattern). Similar report was also unveiled by Luskova et al. (2002) when they exposed *cyprinus carpio* to diazinon for 96 hours.

Decline in values is a clear indication of enzyme inhibition in the kidney of *Heterobranchus bidorsalis*. According to Ovuru and Mgbere (2000), decline in concentration of alkaline Phosphatase activity in organs of fish may not have been impaired. This is because alkaline Phosphataseis known to occur in the cell membrane and may be involved in metabolic transport (Edquist et al., 1992). Hence, the decline in in this study may denote retrogression in metabolic transport. Sasty and Sharma (1980) reported decreased activities of alkaline and acid phosphatase in the brain of *Channa punctatus* following the effect of diazinon. The authors further observed that the alkaline phosphatase activity was inhibited after 96 hours and then it resumes its normal values. Overt decrease in values of alkaline phosphatase in the kidney may depict absolute toxicant effect in the probe organism.

## Conclusion

This study has unveiled the effect of imidacloprid on *Heterobranchus bidorsalis* blood cells as well as enzymes hence the overt effect on these parameters could serve as biomarker in assessment of *Heterobranchus bidorsalis* when exposed to the toxicant. Imidacloprid use should be restricted especially close to aquatic environment. The concentration that elicits changes in the probe organism is much lower than anticipated.

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