



Effect of Imidacloprid on Total Protein, Albumin and Electrolytes in *Heterobranchus bidorsalis*



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Abstract

The aim of this study was to unveil the effects of imidacloprid (a pesticide) on some metabolites (albumin and total protein) and electrolytes (Na^+ , K^+ , Ca^{2+}) in adult *Heterobranchus bidorsalis* (a common Niger wetland fish). Thirty five adult *Heterobranchus bidorsalis* (mean length, 22.43 ± 2.42 cm; mean weight, 166.70 ± 0.33 g) were acclimatized to laboratory condition for seven days and then exposed to varying sublethal concentrations of the toxicant (0.28 , 0.42 and 0.56 mg l^{-1}) in a semi-static bioassay for 14 days. Albumin and total protein were determined in the liver while electrolytes were determined in the gastrointestinal tract (GIT). Results showed that total protein were $5.00 \mu\text{g l}^{-1}$ at 0.00 mg l^{-1} and $33.50 \mu\text{g l}^{-1}$ at 0.56 mg l^{-1} , albumin content were $4.00 \mu\text{g l}^{-1}$ at 0.00 mg l^{-1} and $13.00 \mu\text{g l}^{-1}$ at 0.56 mg l^{-1} , Sodium were $20.50 \text{ mmol l}^{-1}$ at 0.00 mg l^{-1} and $18.50 \text{ mmol l}^{-1}$ at 0.56 mg l^{-1} , potassium $13.05 \text{ mmol l}^{-1}$ at 0.00 mg l^{-1} and 8.60 mmol l^{-1} at 0.56 mg l^{-1} , and calcium were 0.25 mmol l^{-1} at 0.00 mg l^{-1} and 0.30 mmol l^{-1} at 0.56 mg l^{-1} . There was significant variation ($P < 0.05$) among the various concentrations of the toxicant for each of the parameters except for calcium. Total protein and albumin values increased as the concentration of imidacloprid increased (in a dose dependent pattern). Sodium and potassium electrolytes values decreases down the experimental group, but not in dose dependent pattern. Albumin and total protein are useful biomarker of sub-lethal effects of imidacloprid than electrolytes. Additionally, the use of imidacloprid close to aquatic environment should be done with caution.

Keywords: Biochemical metabolites, Electrolytes, Fish, Imidacloprid, Toxicant

Introduction

Pesticide and other xenobiotics present in the aquatic environment can cause several physiological abnormalities and mortality in most organisms living in such ecosystem. Despite pesticide role in enhanced yields and productivity, it has negative ecological consequences in the environment [1]. Contamination by pesticides in the aquatic ecosystem can pose a serious threat to organisms like fish, which are exposed to most of these xenobiotics through anthropogenic depositions [2-11]. In most aquatic environment, studies have shown that fishes are the non-target organisms mostly affected by pesticides [2-7,12]. Imidacloprid is a systemic chloronicotinyl insecticide that enters the target pest through injection or direct contact [13]. Imidacloprid has the potential to disrupt nicotinic acetylcholine receptors in the insect central nervous system [14]. Typically, Imidacloprid is used in the treatment of seeds, soil, crops and control of domestic pests. According to Flores Céspedes et al. [15], imidacloprid affect non-target organisms such as honey bees, ground beetles and fishes. The authors further stated that, these organisms may adversely affected by sublethal doses of the insecticide, but the effect vary widely depending on application method and route of intake.

Contamination of water by pesticides either directly or indirectly can kill fish, reduced fish productivity or elevated concentration of undesirable toxicants in fresh water edible fish tissue which can greatly affect the health of humans consuming these fishes [16]. Fish responds to oxidative stress by evoking

the enzymatic defense system within the body [17]. Pesticide are known to produce many physiological and biochemical changes in aquatic organisms by influencing the activities of several enzymes and metabolites [2-7,18]. Pesticide effect on electrolytes and total protein has been reported [3,4,9,18,19]. A decrease or increase in protein values as a result of exposure of fish to pesticide is an overt indication of physiological imbalance in the fish.

Biochemical indicators of environmental contamination such as enzymes, electrolytes and proteins may be sensitive and early warning indicators of short- or long-term detrimental effects of xenobiotics [20]. Metabolism can be described as the collective term for the chemical process that gives life [21]. Information on the energy metabolism and the factors that influence it is crucial to stress management and handling of fish [21]. The present study contributes to the assessment of Imidacloprid toxicity on some metabolites and electrolytes in *Heterobranchus bidorsalis* (a common Niger Delta wetland fish).

Materials and Methods

Experimental stock

Fish samples (adult *Heterobranchus bidorsalis*) for this study were obtained from a decent private farm at Yenagoa, Bayelsa State, Nigeria. They were transported to the wet laboratory of the Department of Biological Sciences, Niger Delta University, Bayelsa State, where the assays were conducted from January to March

2017. Thirty five (35) adult *Heterobranchus bidorsalis* (mean weight, 166.70±0.33g and mean length, 22.43±2.42cm) were acclimatized individually in a rectangular aquarium for seven days during which they were fed once a day (9.00-11.00hrs) with 35 crude protein at 1% biomass.

General bioassay techniques

Sublethal concentrations of imidacloprid (2.5EC) for the assay (0.28mg/l, 0.42mg/l, 0.56mg/l) were determined based on the range finding test [18]. These were prepared by transferring 0.33mls, 0.53mls, and 0.67mls respectively of the original concentration of the toxicant and making it up to 25L with borehole water on the test aquaria. 25L of the diluents (borehole water) was used as control. Fishes were introduced individually into each aquarium. The exposure period lasted for 14 days during which the exposure media were renewed every forty-eight hours. The physiochemical characterization of the water used for fish bioassay was carried out using standard methods [22] and the following values were obtained: Temperature (26.09-26.04 °C), pH (6.15-6.19), alkalinity (13.35-15.19mg/l), conductivity (.97-118µs/cm) and turbidity (0.49-0.53NTU).

After 14 days exposure period, fishes were killed for collection of samples for analysis via intestine (for electrolytes) and liver (for total protein and albumin). 0.5g of each sample was macerated (grounded) with pestle and mortar [23]. Samples for electrolytes were preserved in deionized water while metabolic samples were preserved using perchloric acid. Samples were centrifuged at the rate of 3000rpm for 15 minutes, the supernatants were then removed and stored in plain bottles at -20°C prior to analysis [23].

Metabolites and electrolytes analysis

Total protein and albumin were determined according to Tietz [24] and Doumas et al. [25] respectively, while APHA [22] method was used for all the electrolytes (Na⁺, K⁺, Ca²⁺).

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 difference (p<0.05) between treatments [26].

Result and Discussion

Table 1 presents total protein and albumin in the liver of *Heterobranchus bidorsalis* exposed to imidacloprid for 14 days. The total protein was 5.00µg/l at 0.00mg/l, 10.25µg/l at 0.28mg/l, 20.50µg/l at 0.42 mg/l and 33.50µg/l at 0.56mg/l. There was significant elevation in total protein as the concentration of toxicant increased (Table 2). Typically, plasma proteins, which include globulins, fibrinogens and albumins, play essential role in transporting materials from one part of the fish to another via the circulation [18]. The findings of this report are contrary to the work of Inyang [18] that exposed *Clarias gariepinus* to diazinon (a well-known organophosphate insecticide). The author also unveiled a gradation: decline of values as the toxicant concentration increased. Increase in values of total protein is attributed to high energy demand in fish due to xenobiotics activities on the tissues of the fish. Due to the low carbohydrate level, protein which is the main architecture of fish cells and main source of nitrogenous metabolism is used to enhance energy demand [10,26,27]. The increase in protein concentration as the concentration of the toxicant increases suggests an interference in protein metabolism.

Table 1: Total protein and albumin in the liver of *Heterobranchus bidorsalis* exposed to imidacloprid for 14 days.

Conc. of Imidacloprid (mg/l)	Total Protein (Tp) (µg/l)	Albumin (Al) (µg/l)
0.00	5.00±0.001 ^d	4.00±0.00 ^d
0.28	10.25±0.03 ^c	6.12±0.02 ^c
0.42	20.5±0.12 ^b	9.01±0.01 ^b
0.56	33.50±0.09 ^a	13.00±0.21 ^a

Means within column with different superscript are significantly different (p<0.05).

Table 2: Electrolytes (Sodium Na⁺), potassium (K⁺) and calcium (Ca²⁺) in the Gastro intestinal tract (GIT) of *Heterobranchus bidorsalis* exposed to imidacloprid for 14 days.

Conc. of Imidacloprid (mg/l)	Sodium (Na ⁺) mmol/l	Potassium (K ⁺) mmol/l	Calcium (Ca ²⁺) mmol/l
0.00	20.5±0.02 ^a	13.05±0.09 ^b	0.25±0.01 ^a
0.28	17.5±0.01 ^c	18.70±0.10 ^a	0.31±0.0 ^a
0.42	15.0±0.03 ^d	9.55±0.10 ^c	0.25±0.02 ^a
0.56	18.5±0.02 ^b	8.60±0.03 ^c	0.30±0.03 ^a

The albumin content was 4.00µg/l at 0.00mg/l, 6.12µg/l at 0.28mg/l, 9.01µg/l at 0.42mg/l and 13.00µg/l at 0.56mg/l, being significantly different (P<0.05). Typically, there was significant elevation in albumin content as the concentration of toxicant increased. A rise in albumin values may be attributed to increase in protein synthesis due to increased enzyme activities involved in protein synthesis [28]. Typically, albumin plays essential role in exerting an osmotic potential which opposes the hydrostatic

pressure developed in blood vessels [29]. Probably due to the antagonistic effect of the toxicant, albumin is needed by fish system for the removal of molecules such as calcium, bile, salts and some steroid hormones in the blood.

The electrolytes (calcium, sodium and potassium) showed fluctuation in the various concentration. Potassium and sodium values fluctuate down the experimental group (not in a dose

dependent pattern). However, Sodium were 20.50mmol⁻¹ at 0.00mg/l and 18.50mmol⁻¹ at 0.56mg/l, potassium 13,05mmol⁻¹ at 0.00mg/l and 8.60mmol⁻¹ at 0.56mg/l, and calcium were 0.25mmol⁻¹ at 0.00mg/l and 0.30mmol⁻¹ at 0.56mg/l. There was significant variation ($P < 0.05$) in the electrolytes concentration except for calcium that were not significantly different ($P > 0.05$).

The basic function of electrolytes in fishes lies in controlling fluid distribution, inter and extra cellular acido-basic equilibrium, maintaining osmotic pressure of the fluids and normal neuromuscular irritability [30]. Several authors have reported the effect of pesticides on fish electrolytes [5,6,9,18,23,31]. Stabilization in values (GIT calcium) could be a stress induced response of fish to toxicants which may have activated certain physiological and processes that could lead to a rapid uptake of the electrolyte from water, food material and a possible reduction of ion efflux [32]. Erhunmwuse & Aninerua [33] reported that sodium is the main regulator of osmotic pressure of the body fluid, and it also initiates and maintains the contraction of heart and involuntary muscles and excites the nerves. Sodium and potassium are essential for acid-base balance and osmotic pressure of the body, while potassium and calcium are essential for neuromuscular excitability [34], hence decline in values of these electrolytes could impact on the cardiac roles of the fish system and general physiology since tissues, organs and systems work together for the general wellbeing of the organism [23].

Conclusion

We conclude that total protein and albumin in the liver could serve as a biomarker for evaluating effect of xenobiotics in *Heterobranchus bidorsalis*. Additionally, the use of this xenobiotics close to aquatic environment should be done with caution.

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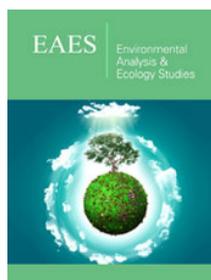
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