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Mini Review

Sharpshooter (40%Profenofos and 4% Cypermethrin)-Induced Oxidative Stress Response in African Catfish Clarias Gariepinus

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Abstract

This study was aimed to determine the effect of exposure of juvenile fish Clarias gariepinus (250 ± 1.2 g) to sub lethal doses (0.014 mg/L and 0.036 mg/L) of sharpshooter on the oxidative stress indices; lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase (CAT) activities over a period of 15 days exposure in three replicates. The colorimetric analysis of the samples collected on day 1, 5, 10 and 15 for LPO, SOD and CAT showed different effects with time and concentration. The LPO at 0.014mg/L and 0.036mg/L were (8.69 ± 4.002^{b2} - 8.390 ± 4.426^{b2}) and (7.275 ± 6.567^{b2} - 7.275 ± 2.00^{b2}) showing increase with increasing concentration of pesticide compared with the control (3.030±0.00b2-1.515±2.142b2) respectively. The SOD activity increased (0.317 ± 0.129^{b2}-0.433 ± 0.055^{b2}) at 0.036 mg/L with time of exposure and decreased with concentration compared with control (0.491±0.006b2-0.053±0.005b2) respectively. CAT activity over the exposure time of 15 days showed a non - significant decrease from 0.16±0.000b2-0.013±0.00b2 at 0.014 mg/L and 0.014 ± 0.000^{b2}–0.011 ± 0.00^{b2} at 0.036 mg/L of sharpshooter respectively compared with control (0.013 ± 0.000^{b2}). At days 5 and 10, sub lethal concentration of sharpshooter (0.036 mg/L) gave significant increase in LPO and decrease in SOD and CAT compared with the control respectively. The result suggests that induction of lipid peroxidation and alteration in the antioxidant enzymes (Superoxide dismutase and catalase) due to the presence of sharpshooter may cause an imbalance in the generation of free radicals and the antioxidants system in juvenile catfish especially at higher concentrations with long exposure.

Keywords: Toxicity; Sharpshooter; Clarias Gariepinus; Oxidative Stress

Introduction

Pesticide is any substance or mixture of substance used to prevent, destroy, or controlling pest which include vectors of human or animal diseases, unwanted plants or animals that cause harm or interfere with the agricultural processes [1]. Pollution of the aquatic environment is one of the major environmental threats in the world as it affects aquatic organism and even health of human being. Frequent discharge of industrial and agricultural wastes into most river results in pollution which could generate various histological, pathological as well as biological alterations in fish [2,3]. The ability of the organisms to develop resistance to most of the insecticide [4] gave rise to the use of mixtures and rotation of insecticides which are reported to be very effective in enhancing the toxicity of insecticides in different resistant pests strain worldwide [5,6]. Sharpshooter is a spectrum insecticide made up of 40% profenofos and 4% cypermethrin as active ingredients. It is used in treatments of ectoparasitic disease and pest of cotton, maize and vegetables [7].

When the production of reactive species overwhelm the antioxidant mechanism in cellular systems, oxidative stress arises damaging the cells [8]. Water pollution contributes greatly to oxidative stress in fish [9]. Xenobiotics like pesticides, agricultural wastes, heavy metals and oil pollutants induce reactive oxygen species through several biochemical mechanisms which results in lipid peroxidation, alterations of cellular redox status and certain aging disease conditions [10-12]. Fish as a bio-indicator of environmental pollution play important role in determining

potential risk associated with contaminated aquatic environment which are directly exposed to chemicals resulting from agricultural production due to surface run-off or indirectly through food chain of ecosystem [13]. In this work we aim at determining the effect of sharpshooter (mixture of 40% profenofos and 4% cypermethrin) on the oxidative stress of juvenile Clarias gariepinus.

Clarias gariepinus is a catfish of the Claridae family found in fresh water, lakes, rivers and swamps and human made habitats. It is found in Africa, the middle East, Brazil and Indonesia. The adult fish measures up to average length of 1-1.5m and weigh up to 60kg with flat body head, broad terminal mouth with four pairs of barbels and large accessory breathing organs made up of modified gill arches [14].

Materials and Methods

Experimental Fish and Acclimatization

One hundred juveniles of C. gariepinus purchased from Rojenny tourist game village, Idemili LGA. Anambra State, Nigeria was transported to Heildin fisheries laboratory unit in Enugu state, Nigeria in 300 litre capacity plastic containers. Acclimatisation of the fish to laboratory conditions took 14 days during which they were fed with commercial feed (6 mm coppens fish feed for agriculture). The container was cleaned and the water changed every morning during the acclimatisation. The fish was not fed for 48 hours before and during the exposure time. A triplicate set of 10 fish specimen was randomly exposed to different concentrations (0.022, 0.036 and 0.044 mg/L) of sharpshooter (mixture of 40% profenofos and 4% cypermethrin) in 10 litres of dechlorinated and aerated tap water to determine the 96 hour lethal concentration (96h LC50) value. The effect of the sub-lethal concentrations of 0.014, 0.036 and 0.00mg/L (control) on the oxidative stress parameters (lipid peroxidation, superoxide dismutase and catalase) for 1, 5, 10 and 15 days were determined in triplicate with sets of 10 fish based on the LC50 of sharpshooter at 96hours.

Assessment of lipid peroxidation (LPO)

This was estimated using thiobarbituric acid reactive substance assay according to [15]. Homogenate of liver sample (0.1ml) was added to 0.1ml of 150mM Tris-HCl (pH7.1), 1.5mM ascorbic acid and 1mM ferrous sulphate in a final volume of 1ml 10% trichloroacetic acid (TCA) and 2ml of 0.375% thiobarbituric acid were added and kept in boiling water for 15minutes.The content was centrifuged at 3000rpm for 10 minutes and the optical density was measured at 532 and 600nm.

$$LPO = \{[(532-600)/0.066] X 2 X10\} mg/100g$$

Assessment of Superoxide Dismutase (SOD)

1.2ml of solution A (50mM sodium carbonate in 0.1mm EDTA buffer, pH10.8),0.5ml of solution B (96µM NBT) and 0.1ml of solution C (0.6% Triton x-100) were incubated at 37^0C for 10minutes with the reaction initiated by adding 0.1ml of 20mM hydroxylamine HCL (pH6.0). The rate of NBT dye reduced by O2- anion generated due to photoactivation of hydroxylamine HCL was recorded at 560nm for 3 minutes as blank while the SOD activity was determined by adding 0.1ml PMS immediately after addition of hydroxylamine HCL to the reaction mixture, mixed thoroughly and the 50% inhibition in the rate of NBT reduction by SOD present in the enzyme source was recorded at 560nm for 3 minutes [16].

Assessment of Catalase

According to [17,18], the assay mixture used were made up of 2.9ml of 12.5mM H2O2, 0.067M phosphate buffer (pH7.0) and 0.01ml PMS. Distilled water is the blank. The decrease in absorbance/30sec at 240nm was measured for 3 minutes.

$$\text{Catalase Activity (k)} = (2.303/\Delta T)\ \alpha\ (\text{Log } A_1/A_1A_2)\ k/min$$

Statistical analysis

The statistical data were shown as the mean ± sem. The significant differences of the data were analysed using analysis of variance (ANOVA) from SPSS statistical package (version 17).

Results

Lipid peroxidation (LPO) values increased from 3.03 ± 0.00^{b2} to 8.690 ± 4.002^{b2} at 0.014mg/L and 7.275 ± 6.567^{b2} at 0.036mg/L in day1 respectively. At day 15 it increased from 1.515 ± 2.142^{b2} to 8.390 ± 4.426^{b2} at 0.014mg/L and 7.275 ± 2.001^{b2} at 0.036mg/L respectively. The lipid peroxidation was significantly increased at day 5 (13.335 ± 2.001^{a2}) and day 10 (13.735 ± 2.566^{a2}) compared with the control.

The superoxide dismutase (SOD) activity decreased from 0.427 ± 0.132^{b2} to 0.239 ± 0.11^{b2} at 0.014mg/l but increased from 0.317 ± 0.129^{b2} to 0.433 ± 0.055^{b2} at 0.036 mg/L with time respectively. The SOD value decreased with increase in concentration of sharpshooter compared with control (0.491 ± 0.006^{b2} to 0.053 ± 0.005^{b2}).There was a significant decrease in the SOD activities at days 5 and 10 when the concentration of the pesticide increased to 0.036mg/L compared with the control.

Catalase (CAT) value decreased from 0.016 ± 0.000^{b2} to 0.013 ± 0.001^{b2} at 0.014mg/L and 0.014 ± 0.000^{b2} to 0.011 ± 0.000^{b2} at 0.036mg/L in exposed juvenile catfish compared to the control group. The CAT values significantly decreased at days 5 and 10 at 0.036mg/L pesticide when compared with the control. The values with different alphabetic (lower case) superscripts differ significantly ($P < 0.05$) between different exposure periods within the same concentration. Values with different numeric superscripts differ significantly ($P < 0.05$)

Discussion

In this present study the oxidative stress indices in the juveniles of the freshwater fish C. gariepinus exposed to sharpshooter

showed increase in lipid peroxidation as the concentration of the pesticide increased. Free radicals generated reacts with biological macromolecules causing increase in lipid peroxidation, deoxyribonucleic acid damage and protein oxidation with ultimate disturbance in the physiological processes [19]. The primary target of reactive species is the polyunsaturated fatty acids in the cell membrane. This may be enzymatic or non-enzymatic [20]. The lipid peroxidation value decreased from 8.690 ± 4.002^{b2} to 4.445 ± 1.152^{b2} for days 1–10 but increased on day 15 to 8.390 ± 4.426^{b2} at 0.014 mg/L pesticide while at 0.036 mg/L, the value increased from 7.275 ± 6.567^{b2} to 13.735 ± 2.566^{a2} for days 1-10 before decreasing at day 15 to 7.275 ± 2.001^{b2} (Table 1). In previous works, elevated lipid peroxidation in fish exposed to different herbicides [21,22] and other toxicants [23,24] were reported. [25] stated that exposure of B. Regularis to herbicide (Butaforce) and insecticide (Termex) caused decrease in lipid peroxidation indicating inactivation of enzyme.

Table 1: The values of oxidative stress indicators of Clarias gariepinus exposed to different concentration of sharpshooter.

Oxidative stress indicators	Day 1	Day 5	Day 10	Day 15
LPO (Mg/100g)				
Control mg/L	3.03 ± 0.000^{b2}	4.545 ± 2.142^{b2}	3.03 ± 0.00^{b2}	1.515 ± 2.142^{b2}
0.014 mg/L	8.690 ± 4.002^{b2}	2.630 ± 0.282^{b2}	4.445 ± 1.152^{b2}	8.390 ± 4.426^{b2}
0.036 mg/L	7.275 ± 6.567^{b2}	13.335 ± 2.001^{a2}	13.735 ± 2.566^{a2}	7.275 ± 2.001^{b2}
SOD activity				
Control mg/L	0.491 ± 0.006^{b2}	0.496 ± 0.012^{b2}	0.054 ± 0.004^{b2}	0.053 ± 0.005^{b2}
0.014 mg/L	0.427 ± 0.132^{b2}	0.496 ± 0.012^{b2}	0.671 ± 0.141^{b2}	0.239 ± 0.011^{b2}
0.036 mg/L	0.317 ± 0.129^{b2}	0.257 ± 0.014^{a2}	0.202 ± 0.092^{a2}	0.433 ± 0.055^{b2}
Catalase (k/min)				
Control mg/L	0.0131 ± 0.00^{b2}	0.016 ± 0.000^{b2}	0.222 ± 0.070^{b2}	0.013 ± 0.000^{b2}
0.014 mg/L	0.016 ± 0.000^{b2}	0.016 ± 0.000^{b2}	0.017 ± 0.002^{b2}	0.013 ± 0.001^{b2}
0.036 mg/L	0.014 ± 0.000^{b2}	0.014 ± 0.000^{a2}	0.014 ± 0.000^{a2}	0.011 ± 0.000^{b2}

During biochemical reactions, reactive oxygen species (ROS) which include hydrogen peroxide (H_2O_2), superoxide anion and hydroxyl radicals are generated [26] and the antioxidant enzymatic systems protect and help to maintain cellular homeostasis by neutralising the ROS [27]. The activity of superoxide dismutase at the highest sub-lethal concentration of 0.036mg/l sharpshooter increased from 0.317 ± 0.129^{b2} to 0.433 ± 0.055^{b2} just like the control (0.491 ± 0.006^{b2}-0.053 ± 0.005^{b2}) contrary to the SOD value at 0.014mg/l which decreased from 0.427 ± 0.132^{b2} to 0.239 ± 0.011^{b2} within the duration of exposure. Also, the SOD response decreased with increase in concentration see (Table 1). This work agrees with [28], who reported that exposure of deltamethrin to B. Viridis gave increased SOD activity. [29,30] demonstrated that different concentration of pollutants generated excess reactive species which inhibited the enzyme activity or inactivated the antioxidant enzymes. The activity of Catalase decreased with time and concentration of sharpshooter compared with control (Table1). [31] reported that toxicity of compounds to organisms has been shown to be dependent on concentrations, sex, developmental stages and exposure periods. The catabolism of superoxide anion produces hydrogen peroxide which is deleterious to protein structures which may be responsible for the decrease in CAT [32]. Moreover, it is reported that herbicides decrease the CAT response to reactive species by binding to CAT or inhibiting synthesis of CAT [25]. This work agreed with [33] who reported the response of catalase, lipid peroxidation and glutathione on zebrafish exposed to deltamethrin.

In the highest sub–lethal concentration of 0.036mg/L sharpshooter, the LPO significantly increased from 4.545 ± 2.142^{b2} to 13.335 ± 2.001^{a2} at day 5 and 3.03 ± 0.00^{b2} to 13.735 ± 2.566^{a2} at day 10 while the antioxidant enzymes SOD and CAT decreased significantly from 0.496 ± 0.012^{b2} to 0.257 ± 0.014^{a2} at day 5, 0.054 ± 0.004^{b2} to 0.202 ± 0.092^{a2} at day 10 and 0.016 ± 0.000^{b2} to 0.014 ± 0.000^{a2} at day 5, 0.222 ± 0.070^{b2} to 0.014 ± 0.000^{a2} at day 10 compared with the control respectively (Table 1).Significant increase in peroxidation of lipid at days 5 and 10 indicates increased reactive oxygen species production with change in concentration from control to 0.036mg/l sharpshooter. Elevated free radicals may overwhelm the antioxidant enzyme system resulting in oxidative stress. The significant decreased effect of sharpshooter on the SOD and CAT activities compared with the control in days 5-10 exposure may be due to limited capacity of the antioxidants system in fish to neutralize the effects of the free radicals [34] and/or free radical damage on the macromolecules of the fish [32]. This result suggests the onset of oxidative stress due to the overwhelming presence of reactive oxygen species generated from the exposure of catfish to the sublethal concentrations of sharpshooter.

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