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

Nwamba Helen O1, Achikanu Cosmas E2* and Ujah II2

1Department of Applied Biology, Enugu State University of Science and Technology, Nigeria
2Department of Applied Biochemistry, Enugu State University of Science and Technology, Nigeria

*Corresponding author: Achikanu Cosmas E, Department of Applied Biochemistry, Enugu State University of Science and Technology, Ebeano, Agbani, Enugu State, PMB 01660, Nigeria. cosmasachikanu@gmail.com, +2348068528362.

To Cite This Article: Achikanu Cosmas E, Sharpshooter (40% Profenofos and 4% Cypermethrin)-Induced Oxidative Stress Response in African Catfish Clarias Gariepinus. 2020 - 7(5). AJBSR.MS.ID.001189. DOI: 10.34297/AJBSR.2020.07.001189.

Received: December 12, 2019 ; Published: February 27, 2020

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±4.00b2 - 8.390±4.426b2) and (7.275±6.567b2 - 7.275±0.00b2) 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±0.129b2-0.433±0.055b2) 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±0.000b2-0.011±0.00b2 at 0.036 mg/L of sharpshooter respectively compared with control (0.013±0.000b2). 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. Acclimatization 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 acclimatization. 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 0.014mg/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 0.006mg/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 150 mM 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 3000 rpm for 10 minutes and the optical density was measured at 532 and 600nm.

\[ \text{LPO} = \left[ \frac{[532-600]}{0.066} \right] \times 2 \times 10 \text{mg/100g} \]

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°C 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 was the blank. The decrease in absorbance/30sec at 240nm was measured for 3 minutes.

\[ \text{Catalase Activity (k)} = \frac{(2.303/\Delta T) \alpha (\log A_i/A_f)}{\text{sem}} \]

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±0.002 to 8.69±0.0022 at 0.014mg/L and 7.275±0.002 at 0.036mg/L in day1 respectively. At day 15 it increased from 1.515±0.1422 to 8.390±4.4262 at 0.014mg/L and 7.275±2.0012 at 0.036mg/L respectively. The lipid peroxidation was significantly increased at day 5 (13.335 ± 2.0012) and day 10 (13.735±2.5663) compared with the control.

The superoxide dismutase (SOD) activity decreased from 0.427±0.1322 to 0.239 ±0.112 at 0.014mg/l but increased from 0.317±0.1292 to 0.433±0.0552 at 0.036 mg/L with time respectively. The SOD value decreased with increase in concentration of sharpshooter compared with control (0.491±0.0062 to 0.053±0.0059).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±0.0002 to 0.013±0.0012 at 0.014mg/L and 0.014±0.0002 to 0.011±0.0002 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 react 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 to 4.445±1.152 for days 1–10 but increased on day 15 to 8.390±4.426 at 0.014 mg/L pesticide while at 0.036 mg/L, the value increased from 7.275±6.567 to 13.335±2.566 for days 1–10 before decreasing at day 15 to 7.275±2.001 (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 | 4.54±2.142 | 3.03±0.000 | 1.51±2.142 |
| 0.014 mg/L                 | 8.69±4.002 | 2.63±0.282 | 4.445±1.152| 8.390±4.426|
| 0.036 mg/L                 | 7.275±6.567| 13.335±2.001| 13.735±2.566| 7.275±2.001|
| SOD activity               |            |            |            |            |
| Control mg/L               | 0.491±0.006| 0.496±0.012| 0.504±0.005| 0.560±0.004|
| 0.014 mg/L                 | 0.427±0.132| 0.496±0.012| 0.671±0.141| 0.239±0.011|
| 0.036 mg/L                 | 0.317±0.129| 0.257±0.014| 0.202±0.092| 0.433±0.055|
| Catalase (k/min)            |            |            |            |            |
| Control mg/L               | 0.0131±0.000| 0.016±0.000| 0.222±0.070| 0.013±0.000|
| 0.014 mg/L                 | 0.016±0.000| 0.016±0.000| 0.176±0.002| 0.013±0.001|
| 0.036 mg/L                 | 0.014±0.000| 0.014±0.000| 0.14±0.000 | 0.011±0.000|

During biochemical reactions, reactive oxygen species (ROS) which include hydrogen peroxide (H₂O₂), 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 0.036 mg/L sharpshooter increased from 0.317±0.129 to 0.433±0.055 just like the control (0.491±0.006–0.503±0.005) contrary to the SOD value at 0.014 mg/L which decreased from 0.427±0.132 to 0.239±0.011 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 (Table 1). [31] reported that toxicity of compounds to organisms has been shown to depend 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 0.036 mg/L sharpshooter, the LPO significantly increased from 4.54±2.142 to 13.335±2.001 at day 5 and 3.03±0.000 to 13.735±2.566 at day 10 while the antioxidant enzymes SOD and CAT decreased significantly from 0.496±0.012 to 0.257±0.014 at day 5, 0.054±0.005 to 0.202±0.092 at day 10 and 0.016±0.000 to 0.14±0.000 at day 5, 0.222±0.070 to 0.014±0.000 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 0.036 mg/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.
References

1. Erhumwunse NO, Dirisu A, Olomukoro JO (2012) Implications of Pesticide Usage in Nigeria. Tropical Freshwater Biology 21(1): 15-25.

2. Reddy HK, Lee SM (2012) Water Pollution and Treatment Technologies. Journal Environ Anal Toxicol 2:4.

3. Kalavathy K, Sivakumar, AA, Chandrin R (2001) Toxic effect of the pesticide dimethoate on the fish sarotherodon messambicus. J Ecology and Research 2(1-2): 27-32.

4. Heingway J, Ranson H (2000) Insecticide resistance in insects vectors of human disease. Annual Rev Entomology 45: 371-391.

5. Khan HA, Akram W, Shad SA, Lee JJ (2013) Insecticide mixtures could enhance the toxicity of insecticides in a resistant diary population of Musca domestica L. PLoS One 8(4): 609-629.

6. Ashad M (2009) Observed potentiation between pyrethroid and organophosphorus insecticides for the management of Spodoptera litura (Lepidoptera nocticulae). Crop Protec 28(3): 264-268.

7. Osibanjo OA, Bamigbseye OM (1990) Chlorinated hydrocarbon in marine fish and shellfish of Nigeria. Marine pollution bulletin 21(12): 581-586.

8. Weidinger A, Kozlov AV (2015) Biological activities of reactive oxygen and nitrogen species: oxidative stress versus signal transduction. Biomolecules 5(2): 472-484.

9. Yildirim NC, Benzer F, Danabas D (2011) Evaluation of environmental pollution at Munzur River of Tunceli applying oxidative stress biomarkers in Capoeta trutta (Heckel, 1843). J Animal and plant Sci 21(1): 66-71.

10. Seveikova M, Modra H, Slaninova A, Svobodovs Z (2011) Metals as a cause of oxidative stress in fish: A review. Veterinary Medicine 56(1): 537-554.

11. Lushchak VI (2011) Environmentally induced oxidative stress in aquatic animals. Aqua toxinol 101(1): 13-30.

12. Blanco Ayala T, Anderica Romero AC, Pendraza Chaverri J (2014) New insights into antioxidant strategies against panququat toxicity. Free Radicals Research 48(6): 623-640.

13. Pessi C, Otachi EO, Korners W, Avenant-Oldewage A, Jira S F, et al. (2017) Fish as bioindicators for trac element pollution at Munzur River of Tunceli applying oxidative stress biomarkers in Capoeta trutta (Heckel, 1843). J Animal and plant Sci 21(1): 66-71.

14. Sambhu C (2004) African catfish, clariasgariepinus (Burchell, 1822): An ideal candidate for biowaste management. Indian journal of experimental biology 42(12): 1226-1229.

15. Buege JA, Aust SD (1978) Microsomal lipid peroxidation. Methods in Enzymology 52: 302-310.

16. Kono Y (1978) Generation of the superoxide radical during antioxidation of hydroxylamine and an assay for superoxide dismutase. Archives in Biochemistry biophysics 186(1): 189-195.

17. Luck H (1971) In: Bergmeyer HO [Ed.] Catalase, Methods of Enzymatic Biochemistry biophysics 186(1): 189-195.

18. Sinha AK (1972) Colometric assay or catalase. analytical biochemistry 47(2): 389-394.

19. Zhou J, Li J, Ran An, Yuan H, Yu F, et al. (2012) Study on a New Synthesis Approach of Glyphosate. J Agric Food Chem 60(25): 6279-6285.

20. Repetto MG, Ferrarotti NF, Boveris A (2010) The involvement of transition metal ions on iron-dependent lipid peroxidation. Archives of Toxicology 84(2): 255-262.

21. Blahova J, Pihalova L, Hostovsky M, Divisova L, Dobsikova R, et al. (2013) Oxidative stress responses in zebra fish Dianorferi after subchronic exposure to atrazine. Food chemistry and toxicoology 61: 82-85.

22. Modesto KA, Martinez CB (2010) Effects of roundup transport of fish: hematology, antioxidant defenses and acetylcholinesterase activity. Chemosphere 81(6): 781-787.

23. Adeyemi JA (2014) Oxidative stress and antioxidant enzyme activities in the African catfish Claras gariepinus, experimentally challenged with Escherichia coli and Vibrio fishcherry. Fish physiology and Biochemistry 40(2): 347-354.

24. Brandao FP, Rodrigues S, Castro BB, Goncalves F, Antunes SC, et al. (2013) Short-term effects of neuroactive pharmaceutical drugs on a fish species: Biochemical and behavioural effects. Aquatic toxicology (144-145): 218-229.

25. Ejiiibe CO, Nwamba HO, Aini CL, Madu J, Onyishigu GC, et al. (2018) Oxidative stress responses in bufo regularis tadpole exposed to butaforo® and termex®. Journal for fisheries and Livestock production 6(2): 1-5.

26. Chandra K, Syed SA, Abid M, Sweety R, Naja AK, et al. (2015) Protection Against Induced Oxidative Stress, Induced DNA Damage as A Model of Arthritis and In vitro Anti-arthritic Potential of Costus speciosus Rhzoxide Extract. International Journal of Pharmcognosy and Phytochemical Research 7(2): 385-399.

27. Schafer FQ, Buettner GR (2001) Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic Biol Med 30(11): 1191-1212.

28. Radovanovic TB, Nasia M, Krizmanic II, Prokic MD, Gavric JP, et al. (2017) Sub-lethal effects of the pyrethroid insecticides deltamethrin on oxidative stress parameters in green toad (Bufoves viridis L.). Environ Toxicol Chem 36(10): 2814-2822.

29. Sun Y, Yin G, Zhang J, Wang X (2007) Bioaccumulation and ROS generation in liver of freshwater fish, goldfish Carassius auratus under hcr omge no 1 exposure. Environ toxicol 22(3): 256-263.

30. Amaeze NH, Onadeko A, Nwosu CC (2014) Comparative acute toxicity and oxidative stress responses in Tadpoles of Amietophryus regularis exposed to refined petroleum products, unused and spent engine oil. African journal of Biotechnology 13(45): 4251-4258.

31. Pandey AK, Nagpure NS, Trivedi S, Kumar R, Kushwaha B, et al. (2011) Profenofos induced DNA damage in freshwater fish Channa punctatus (Bloch) using alkaline single cell gel electrophoresis. Mutational Research 726(2): 209-214.

32. Puerto M, Pichardo J, JosA (2010) Differential oxidative stress responses to microcystin -LR, microcystin - containing and non - containing cyanobacterial crude extracts on Caco-2 cells. Toxicology 55: 514-522.

33. Sharma DK, Ansari BA (2013) Effects of deltamethrin on cat, lpo ang gsh to microcystin -LR, microcystin - containing and non - containing. Toxicology 84: 255-262.

34. Dabas A, Nagpure NS, Kumar R, Kushwaha B, Kumar P, et al. (2011) Assessment of tissue specific effect of cadmium on antioxidant defense system and lipid peroxidation in freshwater murrel, Channapuntatus. Fish physiology and Biochemistry 38(2): 469-482.