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Toxic Effect of Dichlorvos-Pesticide on Lipid Peroxidation, Superoxide Dismutase and Catalase of *Clarias gariepinus*

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Abstract

The sub lethal dose effect of dichlorvos a pesticide (21cand 43 mg/L) on the lipid peroxidation, superoxide dismutase and catalase of juvenile *Clarias gariepinus* (200.15 g) was investigated 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 lipid peroxidation, superoxide dismutase and catalase showed significant increase in a time and concentration dependent manner. The lipid peroxidation increased from (6.05 ± 0.07^{a1}-19.70 ± 10.74^{b1}) at 21 mg/L of dichlorvos and (3.00 ± 0.00^{a1}–51.5 ± 54.31^{b1}) at 43 mg/L of dichlorvos when compared with the control (3.01 ± 0.00^{a1}–3.05 ± 0.07^{b1}; superoxide dismutase activity increased from (0.04 ± 0.00^{a1}–0.21 ± 0.00^{b1}) at 21 mg/l and (0.03 ± 0.01^{a1}–0.21 ± 0.01^{b1}) at 43 mg/l, respectively and Catalase activity also increased from 0.01 ± 0.001^{a1}–0.05 ± 0.05^{b1} at both concentrations of 21 and 43 mg/l of dichlorvos respectively. When compared with the control, the superoxide dismutase and catalase activities decreased in days 1 and 5 but increased in days 10 and 15 at both sub lethal concentrations. The result suggests that dichlorvos may induce oxidative stress that may overwhelm the antioxidant system of juvenile catfish especially at higher concentrations with long exposure.

Keywords: Toxicity; Dichlorvos; *Clarias gariepinus*; Oxidative stress

Introduction

The need to feed the worlds increasing population has prompted the use of agrochemicals such as fertilisers and pesticides to increase food production and ensure the continuation of human race [1]. Dichlorvos (2,2-dichloroviyl dimethyl phosphate) an anticholinesterase, with molecular weight of 220.98, is an organophosphate insecticide used on crops and stored products, as an anti-helminthic and a botacide to kill fly larvae [2]. Increased use of pesticides results in the excess inflow of toxic chemicals into the aquatic ecosystem [3]. Suchismita [4] reported that dichlorvos as an insecticide is also toxic to fish and other aquatic organism while Varo et al. [5] stated that though it is used in treating sea lice on salmon farm, it often produces lethal and sub-lethal effect on fish and the zooplanktons.

Oxidative stress is an imbalance between the production of reactive oxygen species and antioxidant mechanism in cellular systems that results in damaging of the cells [6]. Xenobiotics like pesticides induce reactive oxygen species through several biochemical mechanisms which results in lipid peroxidation, alterations of cellular redox status and certain aging disease conditions [7]. Fish serve as bio-indicators of environmental pollution and can play significant roles in assessing potential risk associated with contamination in aquatic environment since they are directly exposed to chemicals resulting from agricultural production via surface run-off or indirectly through food chain of ecosystem [8]. This work aims at determining the effect of Dichlorvos on the oxidative stress of juvenile *Clarias gariepinus*.

Clarias gariepinus is catfish of the Claridae family. They are found in fresh water, lakes, rivers and swamps and human made habitats such as oxidative ponds and urban sewage system. It is found in Africa, the middle east, Brazil and Indonesia. It has average adult length of 1-1.5 m, can weigh up to 60 kg with flat body head, flat than the genus silurus, broad terminal mouth with four pairs of barbels and large accessory breathing organs made up of modified gill arches [9].

Materials and Methods

Experimental fish and acclimatization

One hundred juveniles of *Clarias gariepinus* with mean weight of (200.15 g) were obtained from Rojenny tourist game village, Idemili LGA. Anambra state, Nigeria in 300 litre capacity plastic containers and transported to Heildin fisheries laboratory unit in Enugu state, Nigeria. The fish were acclimatised to laboratory conditions for 14 days and 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 (18, 20 and 22 mg/L) of dichlorvos in 10 litres of dechlorinated and aerated tap water to determine the 96hour lethal concentration (96h LC_{50}) value. Based on the LC_{50} of dichlorvos at 96hours, the effect of the sub-lethal concentrations of 21 and 43 mg/L on the oxidative stress parameters for 1,5,10 and 15 days were determined with sets of 10 fish. Fish in tap water served as the control with (0.00mg/L) of dichlorvos.

Assessment of lipid peroxidation (LPO)

This was estimated using thiobarbituric acid reactive substance assay [10]. Homogenate of liver sample (0.1 ml) was added to 0.1 ml of 150 mM Tris-HCl (pH7.1), 1.5 mM ascorbic acid and 1mM ferrous sulphate in a final volume of 1 ml 10% trichloroacetic acid (TCA) and 2

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ml of 0.375% thiobarbituric acid were added and kept in boiling water for 15minutes. The content was centrifuged at 3000 rpm for 10minutes and the optical density was measured at 532 and 600 nm.

LPO={[(532-600) nm/0.066] ×2 ×10} mg/100g

Assessment of superoxide dismutase (SOD)

1.2 ml of solution A (50 mM sodium carbonate in 0.1 mm EDTA buffer, pH10.8), 0.5 mL of solution B (96 µM NBT) and 0.1 mL of solution C (0.6% Triton x-100) were incubated at 37°C for 10 minutes with the reaction initiated by adding 0.1 mL of 20 mM hydroxylamine HCL (pH 6.0). The rate of NBT dye reduced by O_2^- anion generated due to photoactivation of hydroxylamine HCL was recorded at 560nm for 3minutes as blank while the SOD activity was determined by adding 0.1 mL 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 560 nm for 3 minutes [11].

Assessment of catalase

According to Luck and Sinha, the assay mixture used were made up of 2.9 mL of 12.5 mM H_2O_2, 0.067 M phosphate buffer (pH 7.0) and 0.01ml PMS. Distilled water is the blank. The decrease in absorbance/30 sec at 240nm was measured for 3minutes [12,13].

Catalase Activity (k)=(2.303/Δ T) α (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

Discussion and conclusion

In this present study we demonstrated the toxic effect of dichlorvos in the juveniles of the freshwater fish of *Clarias gariepinus*. Toxicity of compounds to organisms has been shown to be dependent on concentrations, sex, developmental stages and exposure periods [14]. Reactive oxygen species (ROS) which include hydrogen peroxide (H_2O_2), superoxide anion and hydroxyl radicals are generated during biochemical reactions [15] and the antioxidant enzymatic systems protects organisms from the toxic effects of the free radicals and helps to maintain cellular homeostasis by neutralising the ROS [16]. When there is an imbalance between the ROS and the antioxidant system due to excessive generation of the free radicals, cellular oxidative stress develops [17]. Free radicals generated reacts with biological macromolecules causing increase in lipid peroxidation (LPO), deoxyribonucleic acid damage and protein oxidation with ultimate disturbance in the physiological processes [18]. In this study, the data showed that lipid peroxidation significantly increased in a time and concentration dependent manner (6.05 ± 0.07^{a1}–19.70 ± 10.74^{b1}) for 21mg/L of dichlorvos and (3.00 ± 0.00^{a1}–51.5 ± 54.31^{b1}) for 43mg/L of dichlorvos compared with the control (3.01 ± 0.00^{a1}–3.05 ± 0.07^{b1}; Table 1). The elevated values of lipid peroxidation obtained in this study agrees with previous reports in fish exposed to different herbicides [19,20] and other toxicants [21].

The activity of superoxide dismutase (SOD) and catalase (CAT) enzymes that catalyse the conversion of superoxide radical to hydrogen peroxide and hydrogen peroxide to water and molecular oxygen respectively increased significantly with time and concentration. Exposure of the juvenile fish to dichlorvos for days 1-15, increased superoxide dismutase activity from (0.04 ± 0.00^{a1}-0.21 ± 0.00^{b1}) for 21mg/l and (0.03 ± 0.01^{a1} -0.21 ± 0.01^{b1}) for 43mg/l respectively. Catalase activity also increased from 0.01 ± 0.001^{a1}–0.05 ± 0.05^{b1} at both concentrations of 21 and 43 mg/l of dichlorvos. The activity of SOD and Catalase decreased on exposure of the fish at days 1 and 5 to the pesticide when compared with the control. The activity of superoxide dismutase and catalase in the absence of pesticide decreased from 0.51 ± 0.03^{a1}-0.05 ± 0.00^{b1} and 0.03 ± 0.01^{a1}-0.01 ± 0.00^{b1} with time respectively (Table 1).

LPO				
Control	3.01 ± 0.00^{a1}	3.05 ± 0.07^{b1}	3.00 ± 0.00^{b1}	3.00 ± 0.00^{b1}
21 mg/L	6.05 ± 0.07^{a1}	13.60 ± 6.36^{b1}	6.05 ± 4.31^{b1}	19.70 ± 10.74^{b1}
43 mg/L	3.00 ± 0.00^{a1}	3.00 ± 0.00^{b1}	6.05 ± 4.31^{b1}	51.55 ± 4.31^{b1}
SOD				
Control	0.51 ± 0.03^{a1}	0.49 ± 0.01^{b1}	0.08 ± 0.40^{b1}	0.05 ± 0.00^{b1}
21 mg/L	0.04 ± 0.00^{a1}	0.07 ± 0.07^{b1}	0.15 ± 0.19^{b1}	0.21 ± 0.00^{b1}
43 mg/L	0.03 ± 0.01^{a1}	0.02 ± 0.01^{b1}	0.49 ± 0.60^{b1}	0.21 ± 0.01^{b1}
Catalase				
Control	0.03 ± 0.01^{a1}	0.01 ± 0.00^{b1}	0.01 ± 0.00^{b1}	0.01 ± 0.00^{b1}
21 mg/L	0.01 ± 0.00^{a1}	0.01 ± 0.00^{b1}	0.01 ± 0.00^{b1}	0.05 ± 0.05^{b1}
43 mg/L	0.01 ± 0.00^{a1}	0.01 ± 0.00^{b1}	0.01 ± 0.00^{b1}	0.05 ± 0.06^{b1}

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) between different concentration within the same exposure duration.

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

Increased activities of the superoxide dismutase, catalase enzymes and the peroxidation of lipids within the exposure time indicates that the rate of reactive oxygen species production may have increased with change in concentration from control to 43mg/l dichlorvos. Increased production of the free radicals may result to oxidative stress as the antioxidant enzyme system is overwhelmed. Dabas et al. [17] reported that fish has limited capacity of the antioxidants to neutralise the effects of the free radicals. The decreased effect of the pesticides on the enzyme activity when compared with the control in days 1-5 exposure may be due to free radical damage on the macromolecules of the fish. Puerto et al. [22] reported that decreased SOD activity may be attributed to direct damage of protein structure and an increased production of hydrogen peroxide. This result suggests the onset of oxidative damage of macromolecules due to the overwhelming presence of reactive oxygen species generated from the exposure of catfish to the sublethal concentrations of dichlorvos.

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