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RESEARCH ARTICLE

Genotoxicity assessment and oxidative stress responses in freshwater African catfish *Clarias gariepinus* exposed to fenthion formulations

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Abstract

Fenthion is one of the most widely used organophosphate insecticides for the control of many varieties of pests in Nigeria. The genotoxic effect of the pesticide was evaluated in the blood erythrocytes of *Clarias gariepinus* using the micronucleus (MN) test. The oxidative stress parameters were also studied in the liver and gill tissues. Fish were exposed to 2.0, 4.0, and 8.0 mgL^{-1} of fenthion and sampling was done on days 1, 7, 14, 21 and after 7-day recovery. Micronuclei induction was highest (7.55) on day 14 at all concentrations in the peripheral blood cells. Oxidative stress was evidenced by increased lipid peroxidation (LPO). Maximum LPO values of 62.47% and 71.17% were observed in the gill and liver tissues respectively in *C. gariepinus* exposed to 8.0 mgL^{-1} concentration of fenthion. There were alterations in the values of reduced glutathione (GSH), glutathione reductase (GR), glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) during the exposure and recovery periods. The 7-day recovery period was not adequate to eliminate fenthion-induced changes as LPO, CAT, and GR activity remain elevated. However, MN frequency and activity of SOD, GSH, and GPx (except at 8.0 mgL^{-1}) recovered. The present findings give further credence on the integrated use of MN test and oxidative stress parameters in risk assessment of pollutants in aquatic ecosystem.

Keywords

Fenthion, micronuclei, lipid peroxidation, oxidative stress, *Clarias gariepinus*

History

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Introduction

Fenthion (*O*,*O*-dimethyl *O*-[3-methyl-4-(methylsulfanyl)phenyl] phosphorothioate) is one of the most widely used organophosphate insecticides and avicide in agriculture and public health for controlling many sucking and biting pests (Sevgiler & Uner, 2010; Somdare et al., 2015). In animals, fenthion is quickly absorbed into the bloodstream through the digestive tract, lungs, and skin, and undergoes oxidative metabolism, mediated by cytochrome P450 and flavin-containing monooxygenases (Kerem et al., 2007). The *in vitro* and *in vivo* studies demonstrated that fenthion is biotransformed to fenthion sulfoxide and fenoxon in liver microsomes of fish and rats (Kitamura et al., 2003). Fenthion intoxication in animals causes cholinesterase (ChE) inhibition, inactivity, salivation, muscle fasciculation, dyspnea, flaccid paralysis, vomiting, piloerection, exophthalmia, and diarrhea (Muralidharan, 2014). According to Environmental Protection Agency (2003) and Pest Managing Regulatory Agency (2004), all fenthion formulations have been banned in the United States and Canada due to poisoning-related deaths. However, it is still produced in some countries such as China and India and the application of these insecticides is on-going in Nigeria.

The use of pesticides in agriculture has caused great concern among health and environmental scientists since some of these chemicals are eroded by rains and floods to nearby aquatic systems even when applied in restricted areas. This affects aquatic biota especially fish (Farombi et al., 2008; Ndimele et al., 2010). Furthermore, the high solubility of pesticides, repeated application, accidental spillage, discharge from untreated effluents and spray drift, may result to large build-up and thus help in scaling up their aquatic contamination potential (Jordaan et al., 2013). In the water, the molecules of these contaminants may bind to the materials in suspension, accumulate in the sediment or can be absorbed by the aquatic organisms. These chemicals affect not only the physiology and survival of aquatic organisms but can interact with their genetic material which may lead to mutations and/or carcinogenesis (Ansari et al., 2011; Corredor-Santamaría et al., 2016).

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Micronuclei are some extra nuclei bodies that are formed in mitosis from acentric chromosomal fragments or chromosomes that are not included in either daughter nucleus (Ali et al., 2014). Micronucleus (MN) assay is the most widely used technique for detection of mutagenic effects of various compounds and its feasibility in erythrocytes of *Clarias gariepinus* have been established (Mahboob et al., 2013; Vera-Candioti et al., 2013). Several studies have shown that the technique is favored above others such as the comet assay and chromosomal aberration (CA) test as it is highly sensitive, reliable, easy to adopt, rapid, and can be induced over a short period. It does not require the sacrifice of the experimental fish specimen (Kumar et al., 2013; Nwani et al., 2014; Palanikumar et al., 2012). The genotoxicity of fenthion has been a subject of intense study. While Usha-Rani and Sanjeeva-Rao (1991) showed that fenthion was able to significantly increase CA and single cell exchange (SCEs) in cultured human lymphocytes, Vlastos & Ganidi (2004) reported no significant difference in fenthion-treated human lymphocyte cells. Fenthion was not mutagenic in *Salmonella typhimurium*, *Escherichia coli*, and *Saccharomyces cerevisae* (Waters et al., 1980).

Fishes encounter a variety of stresses during their interaction with aquatic environment. Environmentally induced stresses frequently activate the endogenous production of reactive oxygen species (ROS) which may lead to oxidative stress. The ROS at excess level may react with biological macromolecules to increase the level of lipid peroxidation (LPO), DNA damage, and alterations in the activities of antioxidant enzymes including catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), glutathione reductase (GR), and glutathione peroxidase (GPx). Oxidative stress may cause many diseases such as cancer, respiratory problems, and neurological problems (Vieira et al., 2016). Fish and crustaceans are accepted as biomarkers of environmental pollution (Renu & Saxena, 2015) as they 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 (Lakra & Nagpure, 2009). *C. gariepinus* is one of the dominant fish species distributed widely in Africa and other tropical countries where they inhabit calm lakes, ponds, rivers, and swamps in areas that are seasonally flooded. The fish is widely used in aquaculture, available throughout the year, can acclimatize easily to laboratory conditions and hence, an excellent test organism for toxicological studies (Nwani et al., 2014). In recent years, the integrated assessment of genotoxic as well as oxidative stress modulatory effects of pesticides in fishes have received considerable attentions (Ali et al., 2014; Dar et al., 2015; Franco-Bernardes et al., 2015; Kumar et al., 2013; Nwani et al., 2013). However, to the best of our knowledge no studies have been conducted on the simultaneous assessment of genotoxic and oxidative stress effects of fenthion in fish. The application of battery of biomarkers as in the present study is essential in evaluating the real environmental risk of pesticides. The present study was therefore designed to investigate whether exposure to sub-lethal concentrations of fenthion can induce genotoxic damage in the blood erythrocytes and changes in oxidative stress parameters in the tissues of fresh water African catfish *C. gariepinus*.

Materials and methods

Experimental fish specimen and chemical

Freshwater African catfish *C. gariepinus* (Burchell, 1822) (Family: Clariidae, Order: Siluriformes), mean standard length of 27.36 ± 0.23 cm and weight of 197.39 ± 2.34 g were procured from Freedom Fisheries Ltd Nsukka, Enugu, Nigeria. They were transported to the fisheries wet laboratory, Department of Zoology and Environmental Biology, University of Nigeria Nsukka and treated with 0.05% potassium permanganate ($KMnO_4$) for 2 min to avoid any dermal infections. The fish stock was then acclimatized for three weeks in two plastic tanks of 300 L capacity each and fed *ad libitum* daily with commercial available food (Coppens commercial feed, Coppens International Helmond Netherlands) containing 35% crude protein. To maintain hygienic condition and prevent pollution, fecal matter and other waste materials were siphoned off daily. Dead fish were removed with plastic forceps to avoid possible deterioration of water quality. During the period of acclimation, the water in the tanks was renewed daily with well aerated tap water. The feeding was terminated 24 h prior to the range finding and acute toxicity test to avoid interference of feces. For the present study, commercial formulation of fenthion (containing 600 gL^{-1} fenthion as the active ingredients), manufactured by Yufull Industry Co., Ltd China with CAS No. 55-38-9 was used to make the stock solutions.

Experimental design for chronic exposure and tissue preparation

After acclimatization, the 96 h LC_{50} of fenthion on *C. gariepinus* was determined by probit analysis to be 39.97 mgL^{-1}. Based on this value, three different concentrations of fenthion namely, 2.0, 4.0, and 8.0 mgL^{-1} corresponding to 1/20th, 1/10th, and 1/5th of the 96 h LC_{50} of the insecticide respectively were prepared by serial dilution of the stock solution and used for the *in vivo* chronic exposure. A total of 150 fish from the acclimatized batch was used for the chronic exposure. The fish were divided into five groups of 30 fish each without regard to sex. The fish in the first, second and third groups were treated with 2.0, 4.0, and 8.0 mgL^{-1} of fenthion respectively. The fish specimens in the fourth and fifth groups were maintained in water (without test chemical) and cyclophosphamide (4 mgL^{-1}) to serve as negative control (NC) and positive control (PC), respectively. Each treatment group was set in three replicates of 10 fish each in 40 L glass aquaria ($60 \times 30 \times 30$ cm). The fish were exposed for 21 days after which they were withdrawn from the pesticide and kept in dechlorinated water for 7 days to evaluate the recovery potential. One fish from each replicate (in both the treatment groups and control) was sacrificed after anesthetizing with tricaine methsnessulfonate (MS 222) to minimize stress. This was carried out on days 1, 7, 14, 21 during exposure and after 7-day recovery. The blood samples were immediately processed for preparation of blood smears for MN assay after which they were dissected and liver and gill tissues were

removed, quickly rinsed in 0.9% NaCl solution, and homogenized in pre-chilled potassium phosphate buffer (1: 10 W/V, 0.1 M, pH 7.0). One part of the homogenate was used for the estimation of LPO while the other part was centrifuged for 20 min at 10 500 rpm under 4 °C to obtain the supernatant which was stored at 4 °C for enzyme assay. For comparison, MN test was also carried out on samples of NC and PC. The physicochemical properties of the test water was analyzed daily (APHA, AWWA, WPCF, 2005) and the means were as follows: temperature 25.06 ± 1.18 °C, pH 7.06 ± 0.14, dissolved oxygen $7.34 \pm 1.30\ mgL^{-1}$, conductivity 290 μScm^{-1}, and total hardness as $CaC0_3$ $17.24 \pm 1.05\ mgL^{-1}$.

Micronucleus assay

Peripheral blood samples were smeared on clean, grease free frosted glass slides. Slides were fixed in methanol for 10 min, left to air-dry at room temperature and finally stained with 6% Geimsa in Sorenson buffer (pH 6.9) for 20 min. After dehydration through graded alcohol and clearing in xylene, slides were mounted in a mixture of distyrene (polystyrene), plasticizer (tricresyl phosphate), and xylene. Two slides were prepared from each of the two fish selected from each of the sub-lethal concentration of fenthion. From each of the slides, 1000 erythrocyte cells were scored under a light microscope. For proper identification, each MN must have the same color, plane of focus, clearly separated and smaller than one-third of the main nucleus as reported by Nwani et al. (2014). The MN frequency was calculated thus:

$$\mathrm{MN}(\%) = \frac{\text{Number of cells containing micronuclei}}{\text{Total number of cells counted}} \times 100$$

Determination of lipid peroxidation and enzyme activities

LPO was assayed by measuring malondialdehyde (MDA) formation as described by Sharma & Krishna-Murti (1968). Briefly, 1.0 ml of homogenate prepared in KCL solution was incubated at 37 °C for 30 min. Proteins were precipitated by adding 1 ml of 10% trichloroacetic acid (TCA) and then centrifuged at 2000 rpm for 15 min. 1 ml of supernatant was taken as aliquot in a separate tube to which 1 ml of thiobarbituric acid (TBA)-reacting substance solution was added. The tubes were kept in boiling water bath for 10 min. After cooling the tubes, the optical density (OD) was read at 535 nm.

CAT activity was assayed as described by Aebi (1984). Briefly, 10% homogenate was prepared in 0.9% NaCl and centrifuged at 2500 rpm for 15 min. The supernatant was used for assay. A typical reaction mixture containing 1 ml 50 nM potassium phosphate buffer (pH 7.4) and 25 µl sample was added to the mixture. The reaction was initiated by the addition of dichromate acetic acid and 1 ml of H_2O_2. The amount of H_2O_2 that was consumed was determined by recording absorbance of solution at λ240 nm.

SOD activity was determined by measuring the inhibition of autoxidation of adrenaline at pH 10.2 at 30 °C as described by Misra & Fridovich (1972). Assay mixture contained 0.5 ml bicarbonate buffer, 0.5 ml EDTA, 50 µl sample, 1 ml dH_20, and was incubated for 5 min at room temperature. The reaction was started by the addition of 0.3 ml epinephrine and absorbance was recorded at λ480 nm for 3 min.

Activity of reduced GSH was measured according to the method of Brehe & Burch (1976). 0.1 ml of homogenate was taken in a tube to which 0.9 ml of distilled water and 1.0 ml sulphosalicylic acid was added. The contents were mixed thoroughly and then centrifuged at 5000 rpm for 10 min. 0.5 ml supernatant was taken in a tube and similarly, blank and standards were prepared by taking 0.5 ml of distilled water and 0.5 ml of GSH standard respectively. To all the tubes, 4.5 ml of tris buffer and 0.5 ml of DTNB solution were added. After 6 min OD was read at λ412 nm.

GR was estimated by measuring the rate of conversion of NADPH to $NADP^+$ using the method of Tayarani et al. (1989). Briefly, 10% homogenate was prepared in 1.15% KCl and centrifuged at 10 000 rpm for 10 min and the supernatant was used for the assay. A typical reaction mixture containing 0.7 ml phosphate buffer, 0.1 ml oxidized glutathione (GSSG), and 0.1 ml suitably diluted homogenate was incubated at room temperature for 10 min. The reaction was initiated by the addition of 0.1 ml NADPH and absorbance change was recorded for 5 min at λ340 nm.

The activity of GPx was determined by monitoring the rate of NADPH oxidation at 340 nm by the coupled reaction with GR. The specific activity was determined using the extinction coefficient of 6.22 $mMcm^{-1}$ (Lawrence & Burk, 1976). The values were expressed in unit/min/mg/protein.

Statistical analysis

The data obtained were analyzed using the statistical package SPSS 21.0 computer program (SPSS Inc., Chicago, IL). Differences in the parameters between applied test concentrations and durations were subjected to three-way analysis of variance (ANOVA) followed by Duncan's multiple range tests to determine the significant difference at 5% probability level. Results were expressed as means ± standard error.

Results

Induction of micronucleus

MN induction is one of the most frequently used endpoints for genotoxicity testing in fish and other aquatic organisms (Ansari et al., 2009). The results of MN analysis (Figures 1a–c and 2) in erythrocytes of *C. gariepinus* following exposure to the organophosphorus insecticide fenthion indicated concentration and duration-dependent significant induction of MN. The lowest fenthion concentration ($2.0\ mgL^{-1}$) induced MN frequency of 2.8% in the blood erythrocytes of *C. gariepinus* on day 1 but the frequency increased to 4.60% on day 21 in the same concentration. The highest MN frequency of 7.35% was recorded in the fish group exposed to the highest fenthion concentration ($8.0\ mgL^{-1}$) on day 14. There was decline in MN frequency in all the fenthion concentrations after the MN peak on day 14. After 7-day recovery period MN values in the fish previously exposed to $2.0\ mgL^{-1}$ and $8.0\ mgL^{-1}$ decreased to 2.60% and 3.60%, respectively. There were also significantly ($p < 0.05$) higher MN inductions in the fish exposed to the PC as compared to the NC throughout the duration of the experiment.

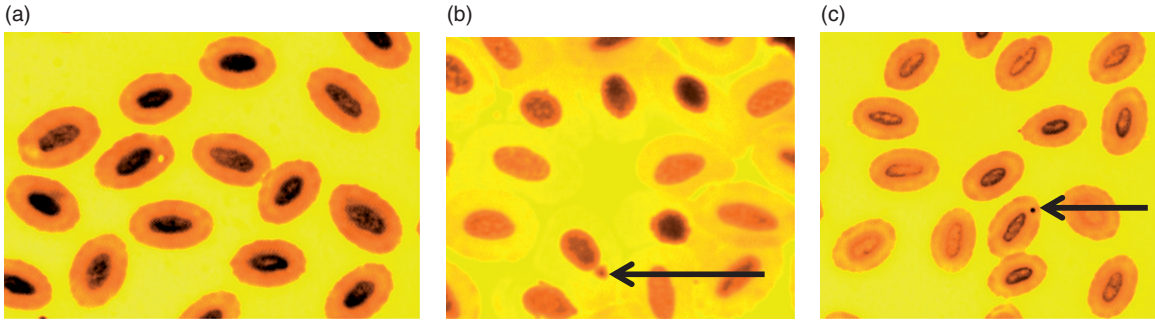
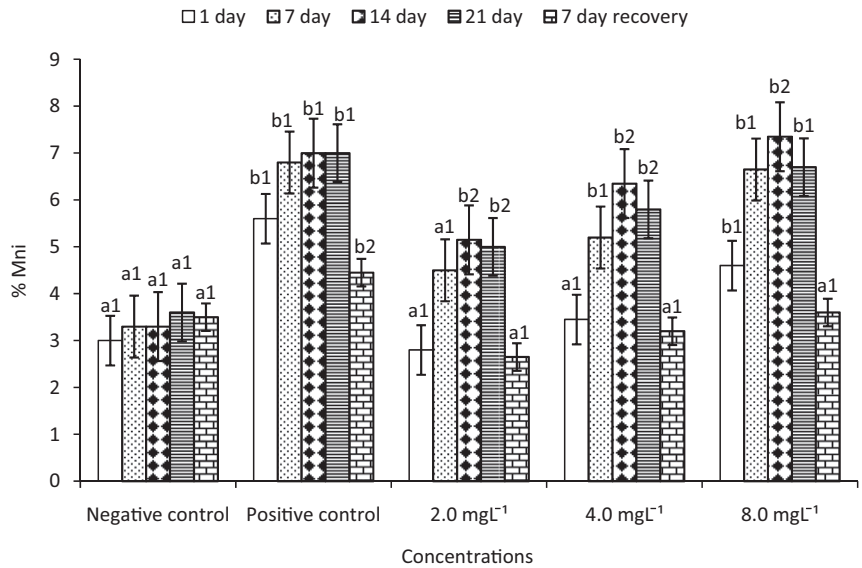


Figure 1. Micronuclei formation in the blood erythrocytes from (a) negative control (NC), positive control (PC) and fenthion-treated peripheral red blood cells of *Clarias gariepinus* after 21-day exposure.

Figure 2. Variation in % MN frequency in blood erythrocytes of *Clarias gariepinus* exposed to fenthion at different concentrations and durations (1, 7, 14, and 21 days and 7-day recovery). Values with different alphabetic (lower case) superscripts differ significantly ($p<0.05$) between exposure durations. Values with different numeric superscripts differ significantly ($p<0.05$) between concentrations within exposure durations.



Effects of fenthion on lipid peroxidation and antioxidant enzymes

The effects of fenthion on tissue membrane LPO measured as the MDA in the liver and gill tissues of *C. gariepinus* is presented in Table 1. There was both time- and concentration-dependent induction of LPO in both tissues of the fish with the lowest MDA formation observed on day 1 of the exposure. At the 8.0 mg/L concentration of fenthion, MDA formation in the exposed fish increased from 13.03% and 21.57% on day 1 to 62.47% and 71.17% on day 21 in the gill and liver tissues of *C. gariepinus*, respectively. During the 7-day recovery period, the values of LPO were higher in the fish previously exposed to the highest concentration of fenthion. At 8.0 mg/L fenthion concentration, the activity of CAT significantly increased ($p<0.05$) from 54.80 and 60.56% on day 1 to 61.05 and 76.21% on day 14 in the liver and gill tissues of *C. gariepinus*, respectively. Compared to the control, GSH activity was significantly elevated in both tissues of *C. gariepinus* up to day 7. GSH activity increased from 4.80% and 5.62% on day 1 to 9.42% and 7.01% on day 7 in the liver and gill tissues respectively at 8.0 mgL^{-1} fenthion concentration. There was however a significant decrease of GSH in all the test concentrations of fenthion from day 14 and 7 days recovery period. The SOD activity of fenthion treated fish at 8.0 mgL^{-1} sub-lethal concentration, showed a biphasic response with an increase on day 14 and decline on day 21 for the gill and liver tissues respectively. SOD values were however comparable to the control during the 7-day recovery experiment. The activities GPx in both tissues of fenthion-exposed fish were reduced throughout the duration of the experiment. Compared to the control, GPx activity reduced from 21.43% and 48.15% on day 1 to 83.33% and 66.67% on day 21 in the liver and gill tissues respectively at the highest fenthion concentration (8.0 mg/L). Sub-lethal fenthion exposure resulted in significantly ($p<0.05$) higher GR activity in both tissues of *C. gariepinus* when compared to the control. GR activity increased from 0.19% to 38.10% and 25.52% to 34.74% in the liver and gill tissues respectively at different time and concentrations of fenthion in *C. gariepinus*. After the 7-day recovery experiment, the values of LPO, CAT, and GR were significantly ($p<0.05$) higher in fish specimen previously exposed to the highest sub-lethal concentration of fenthion (8.0 mg/L) when compared to the control. The GSH activity was however reduced, but SOD values were comparable to the control.

Discussion

Genotoxic assays such as MN test are generally considered useful tools to evaluate the effects of pollutants in fish (Mazzeo & Marin-Morales, 2015) and other aquatic

Table 1. Effects of fenthion on lipid peroxidation and antioxidant enzyme activities in the liver and gill tissues of *Clarias gariepinus*.

Parameter	Tissue	Conc. (mgL^{-1})	Exposure duration (days) 1	7	14	21	7-day recovery
MDA (nmol/protein)	Liver	Control	14.65 ± 3.97^{a2A}	16.81 ± 5.16^{c3B}	19.33 ± 1.72^{b1A}	18.16 ± 1.00^{c2B}	17.57 ± 0.80^{d2B}
		2.0	7.88 ± 0.91^{a1A}	12.45 ± 1.60^{b1A}	16.68 ± 1.44^{b1A}	24.31 ± 2.16^{c1A}	25.52 ± 2.30^{c1A}
		4.0	11.15 ± 0.92^{a1A}	15.97 ± 1.88^{a1A}	19.70 ± 0.94^{a1A}	32.78 ± 0.88^{b2B}	34.44 ± 5.57^{c2B}
		8.0	17.81 ± 0.95^{a2B}	24.57 ± 1.38^{b2B}	30.63 ± 1.20^{c2B}	34.47 ± 1.26^{c2B}	35.35 ± 1.43^{d3B}
	Gill	Control	11.43 ± 0.50^{a3A}	11.46 ± 1.20^{a1A}	17.77 ± 1.62^{b1A}	16.23 ± 0.62^{c2A}	27.56 ± 1.42^{c1A}
		2.0	6.32 ± 0.70^{a1A}	11.30 ± 0.56^{b1A}	16.57 ± 0.55^{c1A}	18.96 ± 0.72^{d1A}	31.67 ± 0.80^{e1A}
		4.0	10.35 ± 0.45^{a2A}	13.61 ± 1.33^{a1A}	17.61 ± 1.55^{b1A}	24.52 ± 1.15^{c2A}	28.58 ± 0.45^{c1A}
		8.0	12.92 ± 1.98^{a2A}	15.37 ± 0.43^{a2A}	25.70 ± 1.25^{b2A}	26.37 ± 1.06^{b1A}	30.75 ± 1.67^{c1A}
CAT (unit/mg protein)	Liver	Control	2.80 ± 0.39^{a1A}	1.07 ± 0.18^{a1A}	1.71 ± 1.89^{a1A}	1.30 ± 0.23^{a1A}	0.97 ± 0.33^{a1A}
		2.0	3.77 ± 0.74^{b1A}	1.20 ± 0.30^{a1A}	2.34 ± 0.65^{ab1A}	1.30 ± 0.41^{a1A}	1.69 ± 0.40^{a1A}
		4.0	4.18 ± 0.24^{b1B}	4.55 ± 0.55^{b2B}	7.07 ± 0.89^{c2B}	1.76 ± 0.12^{a1B}	1.68 ± 0.34^{a1A}
		8.0	7.10 ± 1.41^{b2B}	7.72 ± 1.00^{b3A}	7.19 ± 1.09^{b2B}	2.50 ± 0.60^{a1B}	2.64 ± 0.15^{a2A}
	Gill	Control	2.68 ± 0.25^{a1A}	2.47 ± 0.29^{a2A}	3.03 ± 0.32^{b2A}	2.12 ± 0.43^{a2B}	4.73 ± 0.15^{c2B}
		2.0	2.50 ± 0.14^{b1A}	5.67 ± 0.18^{d2B}	4.21 ± 1.12^{c2A}	0.68 ± 0.10^{a1A}	3.25 ± 0.10^{b1B}
		4.0	3.53 ± 0.07^{b1A}	0.87 ± 0.38^{a1A}	0.92 ± 0.27^{a1A}	1.66 ± 0.06^{a2A}	4.77 ± 0.34^{c2B}
		8.0	5.93 ± 0.77^{b1A}	7.07 ± 0.16^{c3A}	7.78 ± 1.09^{b2A}	1.33 ± 0.18^{a1A}	4.72 ± 0.08^{b2B}
SOD (unit/mg protein)	Liver	Control	1.01 ± 0.02^{a1A}	1.01 ± 0.02^{a1A}	0.94 ± 0.01^{a1A}	1.01 ± 0.00^{a1A}	0.69 ± 0.34^{a1A}
		2.0	1.01 ± 0.01^{b1A}	0.94 ± 0.01^{a1A}	1.42 ± 0.01^{b2A}	1.01 ± 0.02^{c1A}	0.95 ± 0.02^{a1A}
		4.0	0.95 ± 0.03^{a1A}	0.88 ± 0.04^{a1A}	1.45 ± 0.01^{b2A}	1.01 ± 0.00^{c1A}	0.98 ± 0.03^{a1A}
		8.0	0.98 ± 0.01^{ab1A}	0.63 ± 0.26^{a1A}	1.55 ± 0.02^{ab2A}	1.02 ± 0.31^{b1B}	0.98 ± 0.02^{ab1A}
	Gill	Control	0.85 ± 0.15^{a1A}	0.96 ± 0.05^{a2A}	0.93 ± 0.01^{a1A}	0.93 ± 0.01^{b2A}	0.91 ± 0.01^{a1A}
		2.0	0.99 ± 0.01^{a1A}	0.94 ± 0.01^{a2A}	1.04 ± 0.10^{a1A}	0.90 ± 0.04^{a1A}	0.98 ± 0.05^{a1A}
		4.0	0.98 ± 0.01^{b1A}	0.83 ± 0.01^{a1A}	1.14 ± 0.03^{b1A}	1.00 ± 0.00^{c2A}	0.95 ± 0.01^{b1A}
		8.0	0.95 ± 0.03^{a1A}	0.91 ± 0.01^{a2A}	0.96 ± 0.09^{a1A}	0.80 ± 0.00^{a1A}	0.95 ± 0.04^{a1A}
GSH, (mg/protein)	Liver	Control	7.09 ± 0.42^{c2A}	7.02 ± 0.64^{b1A}	4.77 ± 0.43^{a1A}	5.27 ± 0.50^{a1A}	5.77 ± 0.51^{a2B}
		2.0	7.61 ± 1.33^{c3A}	8.33 ± 0.30^{b1A}	4.54 ± 0.30^{a1A}	4.34 ± 0.43^{a1A}	4.99 ± 0.15^{a1B}
		4.0	7.78 ± 0.16^{b1A}	7.80 ± 0.37^{b1A}	4.59 ± 0.27^{a1A}	4.93 ± 0.25^{a1A}	4.48 ± 0.10^{a1A}
		8.0	7.43 ± 0.11^{b1A}	7.75 ± 0.66^{b1A}	4.22 ± 0.19^{a1A}	4.22 ± 0.38^{a1A}	4.20 ± 0.15^{a1A}
	Gill	Control	7.29 ± 0.10^{c2A}	7.97 ± 0.16^{c1A}	4.30 ± 0.31^{a1A}	5.47 ± 0.35^{b1A}	4.60 ± 0.29^{a1A}
		2.0	6.51 ± 0.14^{c1B}	7.71 ± 0.18^{d1A}	4.09 ± 0.23^{a1A}	4.80 ± 0.19^{b1A}	3.63 ± 0.60^{a1A}
		4.0	7.28 ± 0.12^{c2A}	8.13 ± 0.26^{d1A}	4.01 ± 0.01^{a1A}	4.60 ± 0.15^{b1A}	4.02 ± 0.11^{a1A}
		8.0	7.70 ± 0.05^{b3A}	8.57 ± 0.03^{b1A}	4.00 ± 0.12^{a2A}	4.46 ± 0.54^{a1A}	4.58 ± 0.10^{a1A}
GR (nmol/mg protein)	Liver	Control	10.14 ± 0.60^{b3A}	11.39 ± 0.88^{a1A}	12.01 ± 1.16^{a2A}	11.08 ± 0.33^{a1A}	25.23 ± 1.67^{c3B}
		2.0	10.16 ± 0.01^{a1A}	11.03 ± 0.90^{a1B}	10.35 ± 0.89^{a1A}	11.65 ± 0.99^{a1A}	16.03 ± 1.12^{b2A}
		4.0	12.48 ± 0.88^{b2A}	12.13 ± 1.17^{a1A}	13.38 ± 0.68^{b2A}	12.92 ± 1.40^{b1B}	16.94 ± 0.69^{c2A}
		8.0	13.04 ± 0.60^{a1A}	14.40 ± 1.18^{b1A}	18.35 ± 0.67^{c3A}	1 18.12 ± 0.72^{ab1A}	20.60 ± 1.10^{c2B}
	Gill	Control	10.48 ± 1.82^{a1B}	10.58 ± 1.30^{a2B}	10.34 ± 0.34^{a2B}	10.46 ± 0.64^{a2B}	14.99 ± 0.85^{a1A}
		2.0	10.02 ± 0.58^{b1A}	6.40 ± 0.33^{a1A}	10.02 ± 0.59^{b1A}	12.12 ± 0.93^{b2A}	15.13 ± 0.87^{c1A}
		4.0	12.13 ± 1.57^{ab1A}	11.79 ± 0.89^{ab2B}	14.35 ± 0.34^{b2B}	9.62 ± 1.16^{a1A}	14.67 ± 0.49^{b1A}
		8.0	14.07 ± 1.56^{a1A}	14.14 ± 1.02^{a2A}	15.01 ± 0.00^{a1B}	16.03 ± 0.53^{a1A}	16.86 ± 1.46^{b1A}
GPx, (nmol/mg protein)	Liver	Control	0.28 ± 0.07^{a2A}	0.24 ± 0.06^{a2B}	0.39 ± 0.34^{a1A}	0.06 ± 0.03^{a1A}	0.04 ± 0.04^{a1A}
		2.0	0.20 ± 0.09^{b2A}	0.13 ± 0.05^{ab2A}	0.09 ± 0.03^{ab1A}	0.02 ± 0.01^{a1A}	0.01 ± 0.00^{a1A}
		4.0	0.16 ± 0.03^{b2A}	0.02 ± 0.00^{a1A}	0.07 ± 0.02^{a1A}	0.01 ± 0.00^{a1A}	0.01 ± 0.00^{a1A}
		8.0	0.22 ± 0.00^{a1A}	0.28 ± 0.07^{b2A}	0.08 ± 0.02^{a1A}	0.01 ± 0.00^{a1A}	0.38 ± 0.10^{b1B}
	Gill	Control	0.27 ± 0.06^{a1A}	0.16 ± 0.05^{a1A}	0.33 ± 0.30^{a1A}	0.03 ± 0.02^{a1A}	0.01 ± 0.01^{a1A}
		2.0	0.20 ± 0.05^{b1A}	0.16 ± 0.03^{b1A}	0.06 ± 0.02^{a1A}	0.01 ± 0.00^{a1A}	0.01 ± 0.00^{a1A}
		4.0	0.16 ± 0.00^{b1A}	0.20 ± 0.05^{b1B}	0.03 ± 0.00^{a1A}	0.02 ± 0.01^{a1A}	0.05 ± 0.03^{a1A}
		8.0	0.14 ± 0.05^{b1A}	0.17 ± 0.02^{b1A}	0.05 ± 0.01^{a1A}	0.01 ± 0.00^{a1A}	0.01 ± 0.01^{a1A}

Values with different alphabetic (lower case) superscripts differ significantly ($p < 0.05$) between exposure durations. Values with different numeric superscripts differ significantly ($p < 0.05$) between concentrations within exposure durations and tissue. Values with different alphabetic (upper case) superscripts differ significantly ($p < 0.05$) for each concentration between tissues within exposure durations.

organisms (Ansari et al., 2011). Micronuclei can result from elimination of amplified genetic materials from the cell (Fench, 2011) or can result from chromosomal breaks or losses that were not incorporated to the main nucleus during cell division cycle (Renu & Saxena, 2015). In the present study, exposure to sub-lethal concentrations of fenthion resulted in concentration- and duration-dependent increase of MN in the peripheral blood cells of *C. gariepinus* with a peak on day 14. Previous investigations have similarly reported increase in MN frequency in *C. gariepinus* exposed to various pollutants (Ayoola et al., 2012; Mahboob et al., 2013; Mekkawy et al., 2011; Nwani et al., 2014). Furthermore, elevations of MN frequency have also been reported in blood erythrocytes of *Cnesterodon decemmaculatus* (Vera-Candioti et al., 2013), *Channa punctatus* (Chaudhari & Saxena, 2015) and *Rhamdia quelen* (Piancini et al., 2015) exposed to pirimicarb-based formulation, fenvalerate and atrazine herbicide, respectively. Contrary to the present results, Vlastos & Ganidi (2004) reported that fenthion did not induce significant increase in MN frequency in human lymphocytes. The maximum MN frequency observed on day 14 of the exposure may probably be due to oxidative stress resulting from high production of ROS. ROS such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and

hydroxyl radicals (OH^-) are free radicals that contain oxygen atoms that are highly reactive due to the presence of unpaired electrons (Sharma et al., 2014). The inability of the body to eliminate the ROS timely by the antioxidant system would lead to oxidative stress (Dar et al., 2015; Kumar et al., 2013). The ROS produced may cause damage to macromolecules including protein, nucleic acids, and lipids leading to DNA–protein cross-links and DNA damage (Li et al., 2011). It is thus possible that exposure of the fish to sub-lethal fenthion concentrations could cause alterations in the fish cells leading to the formation of micronuclei. The decrease in DNA damage after day 14 of exposure may indicate repair of damaged DNA. Similar repair mechanism was observed in our previous work when freshwater fish *C. punctatus* was exposed to glyphosate-based herbicide (Nwani et al., 2013).

In the present study, the dose- and time-dependent increase in MN frequency was also accompanied by the increase in LPO and changes in the antioxidants. The increase in LPO may be attributed to the ability of fenthion to generate ROS that may interact with the macromolecules of the fish thus resulting to cell damage and alterations of the antioxidants. The fenthion-induced elevation of LPO and subsequent oxidative stress as obtained in the present study is in agreement with Piner et al. (2007) who reported increase in LPO in *Oreochromis niloticus* exposed to fenthion. Increase in LPO leading to oxidative stress have also been reported in rats (Altuntas & Delibas, 2002; Ben Amara et al., 2014; Sefi et al., 2011) and *Rana ridibunda* (Kanter & Celik, 2012) exposed to fenthion. Studies on sub-lethal concentration of deltamethrin in tissues of *C. gariepinus* also revealed elevated levels of LPO in the liver, gill, and kidney tissues of fish (Amin & Hashem, 2012). Dar et al. (2014) reported significant LPO increase in the blood of *Carassius carassius* exposed to endosulfan.

The simultaneous assessment of genotoxic as well as oxidative stress modulatory effects of pesticides as biomarker for evaluating environmental risks have been reported in fish (Altinok et al., 2012; Corredor-Santamaría et al., 2016; Kumar et al., 2013). Raisuddin & Jha (2004) showed that toxicants with oxidative stress have potential to attack DNA resulting in clastogenic and molecular damages. Similar increase in LPO leading to DNA damage and alterations of the antioxidant enzymes were observed in freshwater fish *C. punctatus* exposed to deltamethrin (Ansari et al., 2009), cypermethrin (Ansari et al., 2011), Roundup® (Nwani et al., 2013), and dimethoate (Ali et al., 2014). The cells however have efficient mechanisms of combating the effects of oxidative stress and repair of damaged macromolecules produced during exposure to pollutants. Enzymatic (SOD, CAT, GR, GPx) and non-enzymatic (GSH) antioxidants provide adequate defense and help to scavenge ROS. Under the present study, the values of SOD in both tissues were elevated at the highest fenthion concentration but the values were probably not sufficient to scavenge ROS and combat oxidative stress as consistent increase in LPO was observed during the experiment and recovery period. The activity of CAT and GR was significantly elevated in both tissues of *C. gariepinus* exposed to 8.0 mg/L fenthion. The elevated CAT activity may be in response to the damaging effects of H_2O_2 which results from the degradation of anion superoxide by SOD. The decreased activity of GPx as obtained in our study indicated its reduced capacity to scavenge H_2O_2 and lipid hydroperoxides. Capkin & Altinok (2013) also reported decreased GPx activity in rainbow trout (*Oncorhynchus mykiss*) exposed to carbosulfan. GSH is a nonenzymatic antioxidant that protects the cells from undesirable changes induced by xenobiotics. The increase in GSH levels may be a protective response of cells against fenthion-induced oxidative stress in the fish. Similar increase in GSH levels have been reported in fishes exposed to other toxicants (Dabas et al., 2013; Dar et al., 2014; Stephensen et al., 2002). The decrease in GSH from day 14 may indicate influx of superoxide radicals and the limited capacity of the antioxidant to neutralize oxidative stress as concomitant increase in LPO was observed in all tissues. The higher GR values obtained could be a demonstration that the ability of the enzyme to sustain the recycling of GSSG to GSH was not compromised. The 7-day recovery period was not enough to adequately eliminate the fenthion-induced changes in both tissues of *C. gariepinus* as the values of LPO, CAT, and GR remain elevated. Piner et al. (2007) obtained similar results in *Heteropneustes fossili* exposed to fenthion. However, the MN values and the activity of SOD and GSH recovered and were comparable to the control. GPx values also recovered and were comparable to the control except in the fish earlier exposed to 8.0 mgL^{-1} fenthion. In a similar study, Menezes et al. (2011) reported recovery of SOD and CAT activities in *R. quelen* after 8-day exposure to clomazone. Capkin & Altinok (2013) also reported significant recovery of all biochemical parameters in *O. mykiss* after 21-day post-exposure to carbosulfan.

Conclusion

The present study showed that fenthion not only impose oxidative stress as indicated by increase in LPO and alterations of oxidative stress but also induce micronuclei formation in the blood erythrocytes of *C. gariepinus*. The integrated use of genotoxic and oxidative stress biomarkers using fish model may be useful to the regulatory agencies in risk assessment of pollutants in aquatic ecosystem. Further studies on the toxicokinetics and dynamics of fenthion are necessary for a greater insight on the mechanisms of action that results in induction of oxidative stress and micronuclei formation.

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Declaration of interest

The authors declare that there are no conflicts of interest.

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