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Hepatic oxidative stress, genotoxicity and histopathological alteration in fresh water fish *Labeo rohita* exposed to organophosphorus pesticide profenofos

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ABSTRACT

Organophosphate pesticide profenofos (PFF) is widely used pesticides in agricultural practices throughout the world. Using of various group of pesticide create toxicological and environmental problems, such as impacts on many non-target aquatic species, including fish. This study evaluated the sublethal effects of PFF on the antioxidant enzymes, histopathological alteration and DNA damage in *Labeo rohita.* The lethal concentration (LC50) value of profenofos was 0.1 mg/L for 96 h of exposure. Fingerlings were exposed to two sublethal concentrations 0.02 mg/L (1/5th) and 0.01 mg/L (1/10th) of PFF for a period of 21 days. After profenofos exposure increased SOD and CAT activity was noted only at 7th day but in 14th and 21st day both enzyme activities were significantly decreased. However, GST and LPO activity in liver of exposed group was significantly ($p > 0.05$) increased throughout the study period. In the comet assay, DNA damage in peripheral erythrocytes was enhanced in a concentration and time dependent manner. In addition, histopathological examination in the organs showed significant changes like epithelial lifting, lamellar fusion, epithelial necrosis, in the gill tissue, pyknotic nucleus, vacuolation, nuclear degeneration, cellular edema in the liver tissue and tubular necrosis, shrinkage of glomeruli, hyaline droplets degeneration, vacuolation of renal tubles, pyknotic nucleus in the kidney. The overall results of the present investigation indicated that PFF could potentially induce antioxidant enzyme, DNA damage and histopathological alterations in fish.

1. Introduction

The use of chemical pesticides in agricultural field is fairly well recognized as a cost effective method of controlling pests, but usage of these chemicals resulting in environmental pollution and toxicity to non-target organisms in the environment (Coppage and Bradeich, 1976; Khader Khan, 1996; Shiva Kumar, 2000). Environmental pollution caused by chemical pesticides, has become a serious problem due to their extensive use in agriculture and their persistence. Many of these compounds present in surface and ground waters are considered as potential risk for aquatic organisms as well as water quality (Katsumata et al., 2005; Velisek et al., 2010).

A potential pathway for adverse effects of pesticides is through hydrologic systems, which supply water for both humans and natural ecosystems (Abida Begum et al., 2009). Among different groups of pesticide Organophosphorus compounds (OPs) have been widely used insecticides due to their relatively low persistence under natural conditions and high effectiveness for insect and pest eradication (Qian and Lin, 2015). OPs have been shown to inhibit the enzyme cholinesterase, which functions to rapidly destroy the ubiquitous neurotransmitter acetylcholine (Segall et al., 2003). Among different OPs pesticides profenofos appeared to be the 12th commonest active ingredient in irregular samples of food, being found in samples of orange, strawberry and pepper (ANVISA, 2012).

Profenofos (O-4-bromo-2-chlorophenyl-O-ethyl S-propyl phosphorothioate), is a broad-spectrum organophosphate pesticide used widely for agricultural and household purposes in India (Rao et al., 2003; Rao et al., 2006; Ganguly et al., 2010). It is effective against wide range of chewing and sucking insects and mites on various crops particularly on cotton plants (Chakra Reddyand and Venkateswara, 2008). Its half-life in soil is about one week (Tomlin, 1994). Profenofos is extremely toxic to fish and macroinvertebrates (Åkerblom, 2004a, 2004b). One reason for the extensive use of PFF is because of its short half-life in soil, however, it has been recognized as highly persistent and toxic pesticide even at low concentrations (Zhao et al., 2008).

Fish as bio-indicators of pollutant effects are very sensitive to changes in their environment and play significant roles in assessing potential risk associated with contaminations of new chemicals in

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aquatic environment (Lakra and Nagpure, 2009). The antioxidant defence system has been increasingly studied because of the potential usefulness of oxyradical-mediated responses to provide biochemical biomarkers (Di Giulio et al., 1989; Winston and Di Giulio, 1991). The enzymes that provide the first line of defense include superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR). Lipid peroxidation (LPO) is one of the molecular mechanisms involved in pesticide toxicity (Kehrer, 1993). Alteration of DNA in aquatic organisms has been highly advisable method for evaluating the genotoxic contamination in the environment and various *in vitro* and *in vivo* methods are used to detect the DNA damage in different species (Frenzilli et al., 2009; Tsitsimpikou et al., 2013; Alexander et al., 2016).

Histopathological biomarkers have been widely used in environmental monitoring, as these allow for the examination of specific target organs, including gill, kidney and liver, that are responsible for vital functions, such as respiration, excretion and biotransformation of xenobiotics in the fish (Stehr et al., 2003; Au, 2004; Hinton et al., 2008; Schlenk et al., 2008). This is a non-specific parameter, economical and valuable ecological risk assessment tool (Van der Oost et al., 2003). Hence, a complete systematic evaluation was carried out in the present study by using multiple biomarkers including antioxidant responses, DNA damage and histopathological alterations in fresh water fish *Labeo rohita.*

2. Materials and methods

2.1. Test animal collection and maintenance

The test organisms *Labeo rohita* were procured from Aliyar Fish Farm which is relatively free from pollutants and maintained by the Tamil Nadu Fisheries Development Corporation Limited, Tamil Nadu, and India. Fish were transported with well packed polythene bags containing aerated water. They were acclimatized to laboratory conditions for about 20 days in a large cement tank (containing 1000 L of water). During acclimatization, fish were fed with ad libitum with rice bran, and groundnut oil cake. The water in the tank was renewed daily and was aerated mechanically. These fishes served as the stock for the experimental schedule. The physico-chemical characteristics of the water was maintained as follows. Temperature 25.0 ± 0.5 °C; pH 7.2; hardness 17.8 mg/L (as CaCO3); alkalinity 18.5 ± 7.0 mg/L (as CaCO3) and dissolved oxygen 6.2 ± 0.02 mg/L.

2.2. Test compounds

In the present study profenofos (25% E.C) manufactured by M/s. Syngenta India Limited and supplied by Shakti Agro Service Ltd, Coimbatore, India, was used for evaluation of its toxicity to fish at sublethal level.

2.3. Sublethal toxicity study

For the determination of sublethal toxicity test, a glass tank with 90 l of water was taken. Normal water pH (7.2) was maintained and 1/5th, 1/10th value of the LC50 96 h concentration of profenofos (0.02 (Treatment - l) and 0.01 (Treatment - II) mg/L) was added after removal of same quantity of water. The feeding was discontinued 24 h prior to the experiment. The experiment initiated by introducing 90 fish in a glass tank. Subsequently a common control was also maintained in another glass tank without toxicant. Experiments were conducted for a period of 21 days with 7 days sampling frequency. No mortality was observed during the experimental period.

2.4. Sampling

Upon completion of 7, 14 and 21 days, 20 fish from control and profenofos treated groups were randomly selected, sacrificed without anesthetization and blood from the dorsal aorta was collected using a heparinized syringe which is previously rinsed with heparin. Freshly pooled whole blood was used for genotoxicity studies. At the end of the 21st day gill, liver and kidney tissues were dissected out for the antioxidant and histopathological studies.

2.5. Determination of antioxidant enzymes in liver

The SOD enzyme was assayed according to the method of Marklund and Marklund (1974). The degree of inhibition of auto-oxidation of pyrogallol, at an alkaline pH, by superoxide dismutase was used as a measure of the enzyme activity. The activity of CAT was determined in liver tissue homogenate by the method of Sinha (1972). Glutathione S-transferase conjugates GSH with CDNB and the extent of conjugation is used as a measure of enzyme activity from the proportionate change in the absorption at 340 nm. The levels of lipid peroxidation in liver tissues were determined by the method of Niehaus and Samuelson (1968). Malondialdehyde and other thiobarbituric acid reactive substances (TBARS) are quantitated by their reactivity with thiobarbituric acid (TBA) in acidic conditions.

2.6. Genotoxicity assay

The alkaline comet assay was performed according to Singh et al. (1988) with some modifications. A 30 µL sub-aliquot was taken from diluted blood sample and mixed with 100 µL 0.65% low melting agarose (LMA) in PBS and pipette to fully frosted slides precoated with layer of 100 µL 0.65% high melting agarose (HMA) and slides were kept for 4 h in refrigerator. After solidification, slides were immersed in ice-cold lysing solution and subjected to electrophoresis for 20 min at 25 V (300 mA). After electrophoresis, the slides were neutralized and stained with ethidium bromide. Slides were examined under florescent microscope and images were analyzed by the CASP software.

2.7. Histopathology studies

After treatment, the experimental and control fish were sacrificed at the end of 21st day and gill, liver and kidney tissues were dissected for the histological studies. Tissues were fixed in Bouin's solution and dehydrated through ascending graded series of the ethanol and cleared twice in xylene and embedded in paraffin wax. Sections of the tissues about 5–6 µm were taken by using rotatory microtome and stained with hematoxylin and eosin. Histopathological changes were examined under a light microscope.

2.8. Statistical analysis

SPSS software - Ver.16 statistical package was used for statistical analyses. The significant differences between the control and profenofos treated groups were done by using one way-ANOVA. All statistical analyses based on at $P < 0.05$ significance level.

3. Results

3.1. Acute toxicity test

The 96 h LC50 value of profenofos to *Labeo rohita* was determined as 0.1 mg/L. When fish were exposed to acute (0.1 mg/L) concentration, fish exhibited signs of restlessness, erratic swimming, excessive mucus, and then loss of balance were observed.

3.2. Antioxidant responses

Figs. 1 and 2 shows the effect of profenofos on SOD and CAT, activity in liver tissue of *Labeo rohita*. SOD and CAT activity in liver of