



Quinalphos Induced Antioxidant Status and Histopathological Changes in the Gill of the Freshwater Fish, *Oreochromis mossambicus*

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Abstract: To extend the knowledge about quinalphos induced antioxidant status and its related changes on the histopathology of gills, the freshwater fish *Oreochromis mossambicus* was chosen as a model system. Quinalphos treatment (0.5µl/ L for 30 and 60 days) decreased the activities of antioxidant enzymes with concomitant increase in the production of malondialdehyde. Increased reactive oxygen species generation coincides with the increase in the protein carbonyl in the gills of the treated fishes. Histological observation in the gill of quinalphos treated animal for 60 days showed several alterations as hypertrophy of gill arches, lifting of lamellar epithelium, degeneration of gill filament and lamellar epithelium and vasodilation in the lamellar axis when compared to the control group. These observations suggest that chronic exposure to pesticide affect the respiratory oxidative potential of the freshwater fish and this could be possibly due to quinalphos induced oxidative stress in the gill.

Keywords: Quinalphos; Gill; Antioxidant Enzymes; Protein Carbonyl; ROS; *Oreochromis*.

1. Introduction

The frequent uses of pesticides in agriculture practices as well as in pest control pollute the soil, hydrosol and water bodies thus reaching the aquatic ecosystem to get enriched in the aquatic food chain organisms like fishes. There is considerable information indicating that pesticides are responsible for many adverse effects on fishes and other animals from the histopathological and histochemical points of view. The present work was conducted to study the effect of the quinalphos on the antioxidant status as well as the histopathological changes in the gill of the freshwater fish, *Oreochromis mossambicus*.

The knowledge of oxidative stress in fish has a great importance of environmental and aquatic toxicology because oxidative stress evoked by many chemicals including some pesticides, and the pro-oxidant factors' action on fish organism can be used to assess specific area pollution or world sea pollution. Quinalphos (O, O-diethyl O-*quinoxaline*-2-yl phosphorothioate), an organophosphate pesticide, used extensively in agriculture acts as systemic insecticide

and are widely used as a strong contact and stomach insecticide as well as an acaricide.

Gills are the major respiratory organs and all metabolic pathways depend upon the efficiency of the gills of their energy supply, and damage to this vital organ causes a chain of destructive events, which ultimately lead to respiratory distress (Magare and Patil, 2000). Pesticides can generate reactive oxygen species (ROS) and can lead to histopathological changes, depending on the concentration used, fish species, length of exposure and other factors (Pane *et al.*, 2004). The observation of histological changes in fish gill is a highly sensitive and accurate way to assess the effects of environmental contaminants as quinalphos in field and experimental studies.

Hence, the present study was thus designed to test the hypothesis that elevated ROS generation may contribute to the toxic effect of quinalphos in gill of fishes and the histopathological changes in gill were also observed to see the toxicity of the pesticide. The results of the investigation showed that chronic exposure at a sublethal dose of quinalphos (0.5µl/ L) cause imbalance in the pro-oxidant and antioxidant

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status and this could be a possible reflected reason of histopathological changes in the gills of *Oreochromis mossambicus*.

2. Material and Methods

2.1 Chemicals

Quinalphos (O, O-diethyl O-*quinoxaline*-2-yl phosphorothioate; 70% EC), was purchased from local commercial sources (Product of Hikal Laboratory, Gujarat, India). Malondialdehyde, NADPH and glutathione oxidized, thiobarbituric acid and pyrogallol were obtained from Himedia Laboratories, Mumbai, India. All other chemicals were of analytical grade and obtained from local commercial sources.

2.2 Treatments

The lethal concentration for 50% killing of animals, (LC₅₀) values was computed on the basis of probit analysis (Finney, 1971) for 96 h, which are 5µl/L. In the present study, one-tenth of the dosage (0.5µl/L) quinalphos was chosen to represent sublethal concentration for 30 and 60 days as chronic exposure and respective control animals were maintained. Ten fishes were sustained in every group. Biochemical estimations were done at the end of every treatment and histopathological examinations in gill were performed in both control and treated groups at the end of 60 days.

2.3 Tissue processing and biochemical analysis

At the end of each exposure period, fishes were sacrificed and gills were removed to analyze the biochemical and histological parameters. A 1% (w/v) homogenate of the gill was prepared in ice-cold normal saline with the help of a motor-driven glass Teflon homogenizer on crushed ice for a minute. The homogenate was centrifuged at 8000g for 15 min. at 4°C to obtain the supernatant, which was then used for the biochemical analyses.

Protein was estimated by the method of Lowry *et al.*, (1951) with BSA as the standard. The activity of superoxide dismutase was estimated by the method of Marklund and Marklund, 1974. The activity of catalase was measured by the method of Claiborne, 1985. Glutathione peroxidase was assayed by the method of Mohandas *et al.*, (1984). Levels of lipid peroxidation were measured by the method of Ohkawa *et al.*, 1979. Protein carbonyl was measured as described by Levine (1990).

2.4 Histology

For histopathological preparations, fishes from control and quinalphos treated groups were dissected carefully and gill tissues were removed immediately to prevent autolysis. They were fixed in 10% buffered neutral formalin for 24-48 hours. Tissue was dehydrated in ascending grades of alcohol and was cleared in xylene until they became translucent. Tissue

was transferred to molten paraffin wax for 1 hour to remove xylene completely and impregnated with wax. Then the blocks were cut with a rotary microtome to prepare sections of 5-micron thickness. The sections were stained with haematoxylin and eosin and mounted in DPx. The structural alterations were observed under a light microscope and compared with those of control tissues. Photomicrographs were taken using Cannon shot camera fitted to the Carl Zeiss Axioskop 2 plus Trinocular Research Microscope.

2.5 Statistical analyses

Statistical analyses were performed using one-way analysis of variance (ANOVA) and the means were compared by Duncan's Multiple Range test using statistical package SPSS 17.0. Differences were considered to be significant at $p < 0.05$ against control groups. Data are presented as mean \pm SD for ten animals per group. All biochemical estimations were carried out in duplicate.

3. Result

Chronic exposure to quinalphos in sublethal concentration of 0.5µl/L for 30 and 60 days significantly ($p < 0.05$) decreased the activities of superoxide dismutase, catalase and glutathione peroxidase in the gills of fishes as compared with the corresponding group of control animals (Figs. 1-3). The levels of lipid peroxidation were increased significantly ($p < 0.05$) after 30 and 60 days of quinalphos treatment in gills of the test animal at a time-dependent manner when compared with the corresponding group of control animals (Fig. 4). Increased reactive oxygen species generation coincides with the increase in the protein carbonyl in the gills of the treated fishes (Fig. 5).

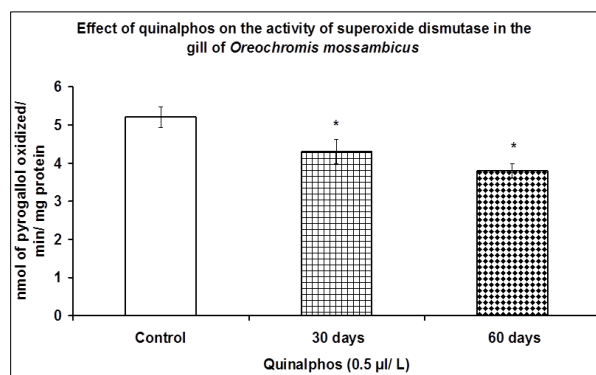


Fig. 1. Effect of quinalphos on the activity of superoxide dismutase in the gill of *Oreochromis mossambicus*. Data are expressed as Mean \pm SD for 10 animals per group. Asterisks (*) denote p values set significant at 0.05 LS against the control group.

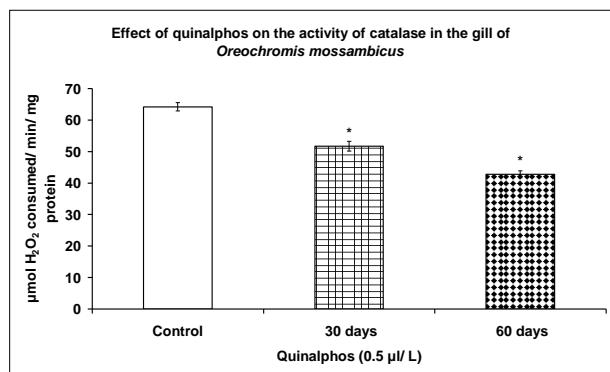


Fig. 2. Effect of quinalphos on the activity of catalase in the gill of *Oreochromis mossambicus*. Data are expressed as Mean ± SD for 10 animals per group. Asterisks (*) denote p values set significant at 0.05 LS against the control group.

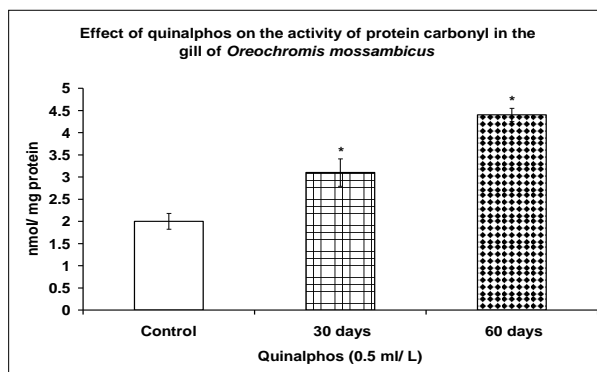


Fig. 5. Effect of quinalphos on the activity of protein carbonyl in the gill of *Oreochromis mossambicus*. Data are expressed as Mean ± SD for 10 animals per group. Asterisks (*) denote p values set significant at 0.05 LS against the control group.

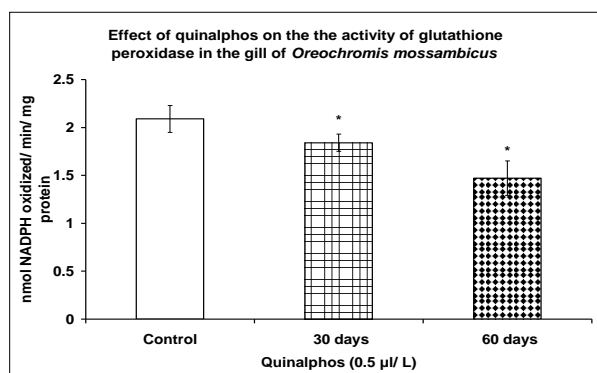


Fig. 3. Effect of quinalphos on the activity of glutathione peroxidase in the gill of *Oreochromis mossambicus*. Data are expressed as Mean ± SD for 10 animals per group. Asterisks (*) denote p values set significant at 0.05 LS against the control group.

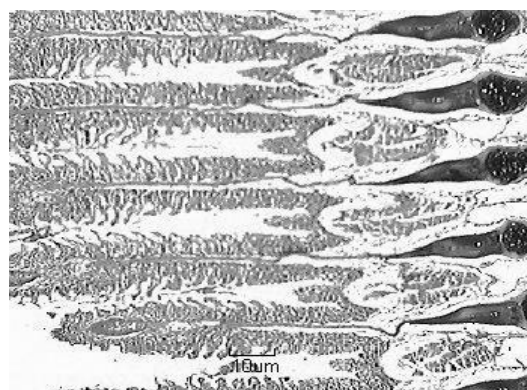


Fig. 6a. Gill arch of control fish (*Oreochromis mossambicus*) showing no histopathological lesions (100 X).

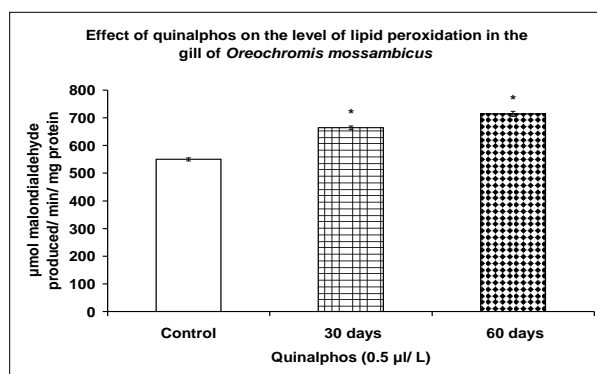


Fig. 4. Effect of quinalphos on the level of lipid peroxidation in the gill of *Oreochromis mossambicus*. Data are expressed as Mean ± SD for 10 animals per group. Asterisks (*) denote p values set significant at 0.05 LS against the control group.

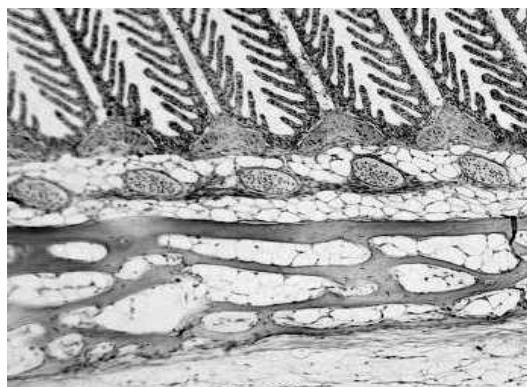


Fig. 6b. Gill arch of control fish (*Oreochromis mossambicus*) showing no histopathological lesions (400 X).

Histopathological study of the gills exposed to quinalphos for 60 days showed several alterations as hypertrophy of gill arches, lifting of lamellar epithelium, degeneration of gill filament and lamellar epithelium and vasodilation in the lamellar axis when compared to control tissue (Figs. 6-9).

4. Discussion

Fishes are relatively sensitive to changes in their environment and they have a relatively long lifespan compared to the other aquatic organisms. These animals can, therefore, give indication of the general health of the specific habitat or aquatic ecosystem in which they live. Of all vital organs, gills are important not only for gaseous exchange but also participate in many other

vital functions in fish such as osmoregulation, ion regulation and excretion of toxic waste products and remain in close contact with the external environment and particularly sensitive to any changes or disturbances in the quality of water (Perry and Laurent, 1993). It is well known that histopathological, biochemical or physiological changes in gill of fishes are among the most commonly recognized responses to environmental pollutants (Mallatt, 1985, Laurent and Perry, 1991). Thus it is expected that any harm in the fish gills leads to impairment of vital organ revealing respiratory distress, impaired osmoregulation, and retention of toxic wastes, asphyxiation, reduced chemoreception or other conditions that can lead to disease or even to death.



Fig. 7. Hypertrophy of the gill arch and complete degeneration of gill filament and lamellae after 60 days of quinalphos treatment (100 X).

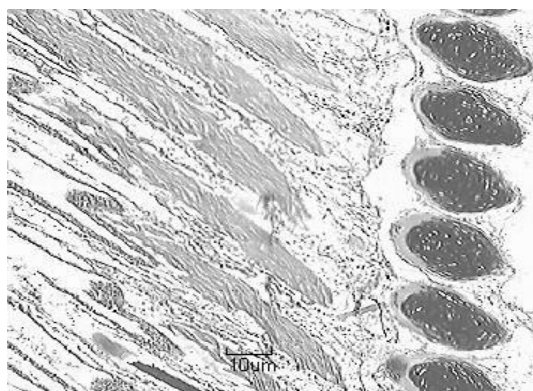


Fig. 8a. Degeneration and vasodilation in the lamellar axis observed after 60 days of quinalphos treatment (100 X).

Pesticides in the environment when enter to the aquatic ecosystem exert their effects through their ability to redox cycle (one electron reduction and oxidation reactions) and the negative side effects include the formation of reactive oxygen species (ROS) with the ability to damage cellular molecules. When the production of ROS in the tissues exceeds the ability of the antioxidant system to eliminate them, oxidative

stress ensues (Sies, 1991). Because of the high reactivity of ROS, most cellular components are likely to be targets of oxidative damage; lipid peroxidation, protein oxidation, GSH depletion and DNA single-strand breaks. As a whole, these events ultimately lead to cellular dysfunction and injury (Marks *et al.*, 1996). The physiological systems of many aquatic organisms, which are in place to metabolize these compounds and resulting byproducts, are evolutionarily similar to those in humans. The present study focuses on the effects of quinalphos on the antioxidant status in freshwater fish, *Oreochromis mossambicus* and its possible role in mediating the changes in the histopathology of gills.

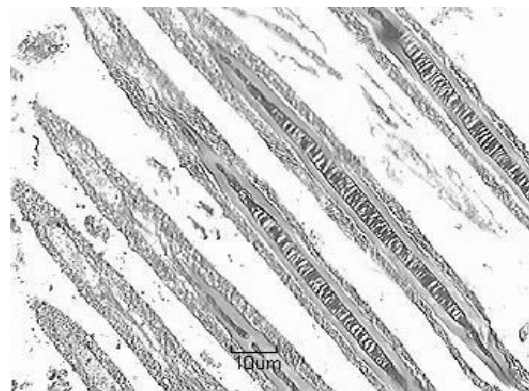


Fig. 8b. Degeneration and vasodilation in the lamellar axis observed after 60 days of quinalphos treatment (100 X).

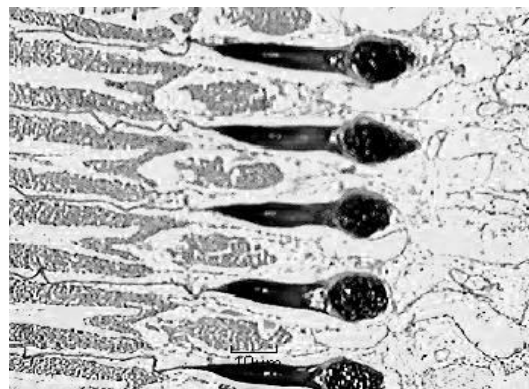


Fig. 9. Uplifting of lamellar epithelium after 60 days of quinalphos treatment (100 X).

The body weights of fishes were monitored throughout the study to ensure the effect of toxic compounds on the general health status of the animals. No significant changes in the body weights were observed in the treated animals as compared with the control groups throughout the experiments (data not shown).

In the present study, quinalphos-treatment for 30 and 60 days resulted in the significant decrease of antioxidant enzymes with concomitant increase in the lipid peroxidation in time-dependent manner when compared with corresponding control groups (Figs. 1-

4). A disturbance in the balance between the prooxidants and antioxidants leading to detrimental biochemical and physiological effects is known as oxidative stress. This is a harmful condition in which increases in free radical production, and/or decreases in antioxidant levels can lead to potential damage to the tissues. Indicators of oxidative stress include changes in antioxidant enzyme activity, damaged DNA bases, protein oxidation products, and lipid peroxidation products.

ROS are also known to convert amino groups of proteins and thereby alter protein structure or function, leading to changes in enzyme activity, receptor function, transport proteins and signal transduction pathways. This disruption can eventually lead to cell death. Protein carbonylation involves the formation of carbonyl moieties (-C=O) at amino acid side chains. An increase in the number of carbonyl groups correlates well to protein damage caused by oxidative stress (Shacter *et al.*, 1994). Malondialdehyde (MDA) contains two aldehyde groups which can react with lysine α -amino groups of proteins to produce carbonyl groups. In more recent years, protein carbonylation has been used as a sensitive biomarker of pesticide effects in fish. In the present study, an increase in protein carbonyl levels could indicate that normal protein metabolism is disrupted, resulting in accumulation of damaged molecules (Fig. 5). This indicates that measurement of protein carbonylation provides a potentially useful biomarker of oxidative stress in fish (Dalle-Donne *et al.*, 2003).

In the present study, the gills of control fish (*Oreochromis mossambicus*) showed a typical structural organization of the primary lamellae and secondary lamellae. Gills are composed of four-gill arches on each side with normal arrangement pattern. Projecting on lateral sides of primary lamellae which is covered by stratified squamous epithelium are the secondary lamellae (respiratory lamellae). The surface of secondary lamellae is covered with a delicate layer of a simple squamous epithelium. Each gill arch contains afferent brachial arteries and efferent brachial arteries covered by epidermal tissue with mucous cells (Fig. 6).

However, in the present study quinalphos treatment pronounced secretion of mucus layer over the gill lamellae. Secretion of mucus over the gill curtails the diffusion of oxygen, which may ultimately reduce the oxygen uptake by the animal (David *et al.*, 2002). Aysel and Koksall (2005) reported that mucous secretion is a symptom of gill necrosis and hypersecretion of mucus observed in the present study represented by a thick coating of heavy mucus secretion on the gill surface is a common protective phenomenon of the fish to prevent entry of quinalphos in the gills. However, the excessive coagulation of mucus over the gills impairs the respiratory function of fishes (Prasad, 1988). If gills would be destroyed due to pesticides or the membrane

functions are disturbed by a changed permeability then also the oxygen uptake rate would even rapidly decrease.

Histopathological study of the gills exposed to quinalphos shows several alterations as hypertrophy of gill arches, lifting of lamellar epithelium, degeneration of gill filament and lamellar epithelium and vasodilation in the lamellar axis when compared to control group (Figs. 7-9). Similar results were observed in the fishes exposed to other pollutants (Figueiredo-Fernandes *et al.*, 2007). In the present study, the histopathological changes observed in gill of fish may be due to quinalphos toxicity and this could be possible owing to oxidative stress induced by quinalphos. It can be concluded that gill of fish may serve as a sensitive biomarker for the toxicity of sublethal concentrations of quinalphos. However, further corresponding studies are necessary for the better understanding of its deleterious effects.

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