Research Article

Assessment of Antioxidant Enzymatic Activities in Liver, Gill and Brain of *Oreochromis mossambicus* on Ethoxyquin Exposure

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ABSTRACT

The present study was undertaken to assess the toxicity of ethoxyquin on antioxidant enzyme system in liver, gill and brain of fresh water fish, *Oreochromis mossambicus*. Ethoxyquin (EQ) is a synthetic antioxidant that is included in some animal and human foods as a preservative to protect fats and fat-soluble vitamins from oxidative degradation. Many unfavourable side-effects have been observed in animals fed with EQ-containing feeds. EQ can alter the biochemical variables and antioxidant defence mechanism and thus injurious to vital organs. The median lethal concentration for 96 hours (LC50-96h) in the fish observed by bioassay analysis was 11.37mg/L. Fishes were exposed to sublethal concentration of EQ ie., 1.137mg/L for 24-96 hours. The effects of EQ on antioxidant enzyme activities –Superoxide dismutase (SOD), Catalase (CAT), Glutathione reductase (GR) and Glutathione peroxidise (GPX) –were analysed at the end of particular exposure period. A significant increase in SOD and CAT activity in all the three organs on EQ exposure was noticed suggesting an elevated antioxidant level in order to neutralise the impact of reactive oxygen species. There was a significant decrease in GR and GPX activity on EQ exposure at different time intervals in all the three tissues. This decrease may be due to increase in oxidative stress which is an indication of impaired antioxidant defence mechanism as a result of excessive generation of free radicals. The alterations of the enzymatic parameters can be effectively used as potential biomarkers for monitoring of the EQ toxicity in aquatic environment.

Keywords: Ethoxyquin, *Oreochromis mossambicus*, Median lethal concentration, Antioxidant enzymes.

INTRODUCTION

The release of toxic contaminants from domestic, industrial and agricultural activities into the aquatic environment leads to serious effect to environmental and health worldwide. Ethoxyquin (EQ; 6-ethoxy-1,2-dihydro-2,2,4-trimethyl quinolin) is primarily registered as a pesticide in 1965 and is broadly used as an antioxidant. EQ has been used for post-harvest indoor application for fruits[1]. The exposure of aquatic organisms to EQ via contaminated water is far less well studied. Irrespective of its extensive global use, knowledge of the occurrence of EQ in surface water is scarce. The toxicity of EQ on aquatic life has rarely been investigated. The occurrence of EQ and its metabolite in edible parts of fish at considerable concentrations led to the assumption that EQ, together with its metabolite, may reach the maximum tolerable threshold value for human consumption[2].

A large number of studies have shown the negative effect of different pollutants on the aquatic biota resulting in the loss of biodiversity and making the water unfit for life. The early detection of harmful changes in the environment is necessary to reverse the degradation of the ecosystem. Biomonitoring, which uses organisms to indicate the health of the environment, is well suited to this purpose as the existing organisms are adapted to the specific ecosystem and any alterations in the environment are reflected in the composition and structure of the biological communities[3,4]. Both invertebrates and vertebrates have been used for bio-monitoring. Since fishes occupy an intermediate range in the food pyramid, they are ideally suited for bio-monitoring.

Fish can serve as bio-indicators of environmental pollution and therefore can be used for the assessment of the quality of aquatic environment since they are directly exposed to chemicals resulting from agricultural production via surface runoff of water or indirectly through the food chain of ecosystem [5,-7]. Liver plays an important role in vital functions of basic metabolism and represents the major organ for the accumulation, biotransformation and excretion of contaminants in fish[8,9]. The gills are the main targets of direct contamination, as they play a significant role in metal uptake, storage and eventually, transfer to the internal compartments via blood transport [10]. Fish typically have quite small brains relative to body size compared with other vertebrates.Its function include postural control,detection of luminance and monitoring of saccadic movement. Analysis of biomarkers in aquatic organisms particularly in fish is a validated approach for early warning of chemical exposure[11,12]. Toxicants may cause an increase in the production of reactive oxygen species (ROS) in aquatic organisms which may lead to impairment of many cellular functions [13-16]. The deleterious effect of free radicals can be prevented or counter balanced by antioxidant systems[17,18]. Fish are endowed with defensive mechanisms to counteract the impact of reactive oxygen species (ROS) resulting from the metabolism of various chemicals. These systems include various antioxidant defense enzymes such

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as superoxide dismutases which catalyze the dismutation of superoxide radical to hydrogen peroxide, catalase acting on hydrogen peroxide, glutathione S-transferase family possessing detoxifying activities towards lipid hydroperoxides generated by organic pollutants such as heavy metals[19]. Glutathione Peroxidase (GPx) is a selenocysteine peroxidise that plays a more crucial role of inhibiting lipid peroxidation process, and therefore protects cells from oxidative stress[20].

Mozambique tilapia, *Oreochromis mossambicus*, has commonly used as a target biological model in toxicology studies. This popular freshwater fish species belonging to the child family has several distinctive features that support its suitability for use as a model species for the assessment of aquatic pollution. It is known by its high growth rates, distinct ability to adapt to diverse diets, great resistance to diseases and handling practices, easy reproduction in captivity and prolific rate of breeding and multiplication,and tolerance to various environmental conditions[21]. In fact,tilapia has previously been used as a sentinel organism for contaminants in various toxicological studies[22]. Alterations in the biotransformation enzymes of this indicator species have also been used in various biomonitoring studies to reflect and assess environmental contaminants.

The liver and gill are the main and main sensitive target organs of xenobiotic toxicity and damage for fish. They also play a major role in the biotransformation of xenobiotics. The sensitivity of these tissues to pesticide-induced stress is a function of the disturbed balance between the degree of oxidative stress and the antioxidant capability[23,24]. Previous studies have shown that pesticides alter enzymatic and nonenzymatic antioxidant systems and induce oxidative stress in animals[25,26]. The oxidative stress-induction potential of exposure to different sublethal concentrations of chlorpyrifos has previously been investigated in the brain, liver and gill tissues of guppy fish, *Poecilia reticulate* [27].

The present study was undertaken to investigate and evaluate the potential biochemical changes in the activity of antioxidant enzymes in the liver,gill and brain of *Oreochromis mossambicus* exposed to sub lethal concentrations of EQ at different time intervals (24-96hr).

MATERIALS AND METHODS

Collection and maintenance of fish

Oreochromis mossambicus were purchased from Aquafish Aquarium fish farm, Kottakal, Malappuram District, Kerala. The average weight of fish was 2 gram and 8 centimetres in length. The fish were kept in the lab for two weeks prior to the experiment, where they were provided with a constant supply of water and a well-lit environment. Fish were fed the recommended quantity of commercial fish pellets three times a day and housed in thirty-litre glass aquariums with plenty of oxygen throughout the acclimation period. Light and dark cycles were managed at a ratio of twelve hours of light to twelve hours of darkness, and the aquarium water was dechlorinated and replaced regularly with new water. Researchers monitored the health of the fish and instantly eliminated those that seemed to be in bad condition from the aquariums. Preliminary screening and standardization of physicochemical properties of tap water, including water temperature (28 to 29°C), pH (6.5 to 7.5), and oxygen saturation (70 and 100%), were carried out in accordance with the criteria established by the American Public Health Association (APHA, 1998). Features such as water temperature (between 28 and 29 degrees Celsius), pH (between 6.5 and 7.5), and oxygen saturation (between 70 and 100 percent) are all taken into account.

The chemicals used were of a toxicant used analytical purity. They were used without being refined further. The toxicant used was Ethoxyquin (EQ) (6-ethoxy-1, 2-dihydro-2, 2, 4-trimethyl quinolin), purchased from Sigma Aldrich, Germany.

Acute toxicity test

The median lethal concentration (semi-static; 96 h-LC_{50}) over a period of 96 hours was determined by applying ethoxyquin concentrations varying from 5-25mg/L to five different groups with 15 fishes each group. The mortality is observed for 96 hrs. Probit Analysis was performed for determining LC_{50} values. Sub lethal doses, comprising one-tenth of the 96 h-LC_{50}, were selected for further toxicological studies based on the median lethal concentration for 96 h duration.

For toxicity induction studies the experimental design was done as follows; Fishes were divided into five groups, with nine specimens in each group. Group I was negative control group (without toxicant). In group II to V sub lethal concentration of 96h -LC_{50} (ie, 1/10th of medial lethal concentration) was added and exposed to various time intervals like 24hr, 48hr,72hr,96hr. After each time of exposure the alteration in antioxidant enzymes Superoxide dismutase (SOD), Catalase, Glutathione reductase (GR) and Glutathione peroxidise (GPX) in the liver,gill and brain of *Oreochromis mossambicus* were assayed. Liver, gills and brain were excised and homogenized in ice-cold 0.25M sucrose buffer, pH 7.4. The homogenate was centrifuged at 5000 rpm for 15 min at 4^0C. The tissue supernatant was used for measurement antioxidant parameters.

Total protein was quantified according to the method developed by Lowry *et al*., (1951) using Folin's reagent, and BSA as a standard. The SOD activity in the tissue extract was assayed by the method of pyrogallol auto oxidation by superoxide radicals and expressed as U/mg protein[28]. Catalase (CAT) activity was determined by monitoring the decrease in absorbance of H_2O_2 at 240 nm and expressed as µmol/mg protein/min[29]. Glutathione reductase (GR) activity was determined and expressed as nmol NADPH oxidized per min per mg of protein[30]. Glutathione peroxidise (GPX) activity was assayed following the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) as a substrate with t-butyl hydroperoxide[31]. The

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activity of GPX was expressed as nmol NADPH/min/mg protein.

Statistical analysis

The experiments were repeated on three different occasions in triplicate and the data were analysed by Student's *t*-test. Statistical comparisons were done between control and exposure data from the same species. A probability level below 0.05was taken as statistically significant.

RESULT AND DISCUSSION

The changes in the activities of antioxidant enzymes SOD, CAT, GPX and GR in the liver, gill and brain of *Oreochromis mossambicus* on exposure to EQ is depicted in fig 1,2,3 and 4 respectively.

It was found that a significant increase in SOD activity in all the three organs on EQ exposure. A significant ($p < 0.05$) increase over control was observed in SOD activity in gill and liver. At 72 hr and 96 hr exposure the increase in SOD activity of gill was found to be high compared to 24hr and 28 hr. In brain too SOD activity was found to be increased.

It was found that CAT activity was increased significantly on EQ exposure at all time of exposure in all the three organs. The increase was more profound in liver and gills compared to brain.

Elevation in the SOD and CAT level indicates an elevated antioxidant level in order to neutralise the impact of reactive oxygen species (ROS). The excessive ROS production and their damaging effects can be minimized by the cellular antioxidant systems[32,33]. The enzymes such as SOD and catalase play a major role in eliminating the ROS produced during bioactivation of xenobiotics and the induction of SOD/CAT system may be the first defense mechanism against ROS. Moreover, SOD and CAT are highly sensitive and respond more quickly thereby protecting organisms from oxidative stress [34-36].

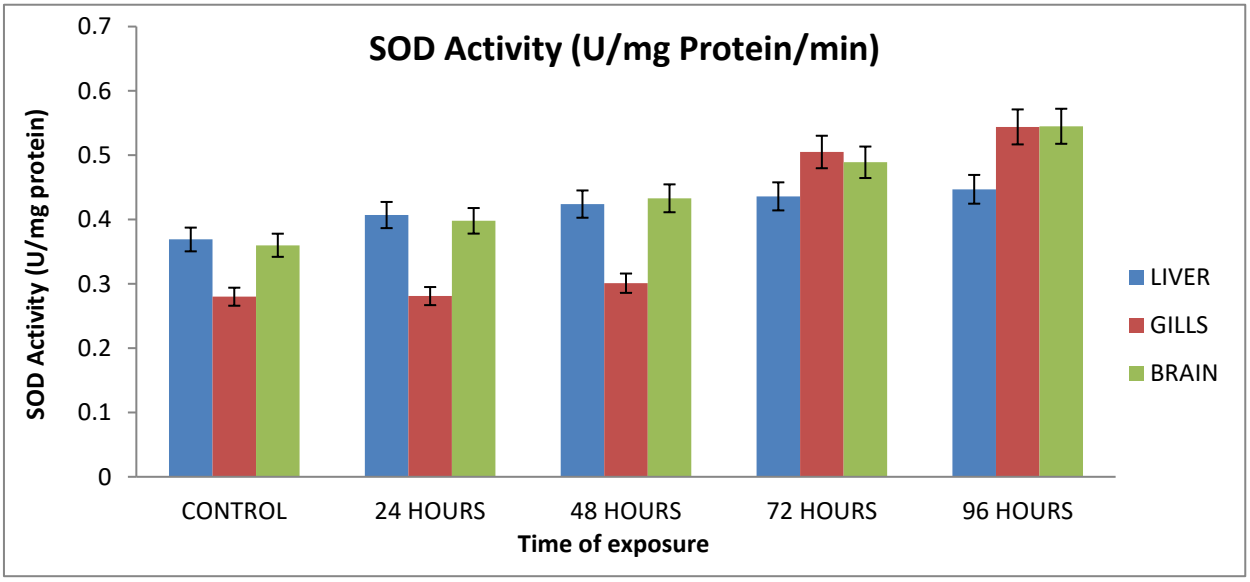

Figure 1: Effect of EQ on SOD Activity in liver, gill and brain of *Oreochromis mossambicus*

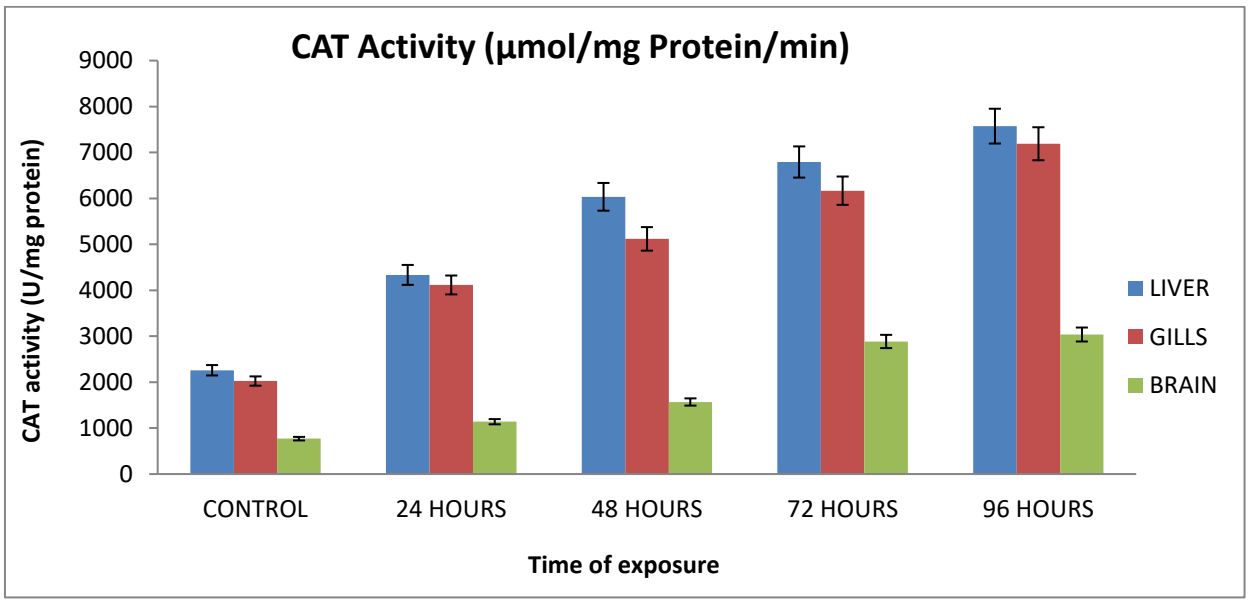

Figure 2: Effect of EQ on CAT Activity in liver, gill and brain of *Oreochromis mossambicus*

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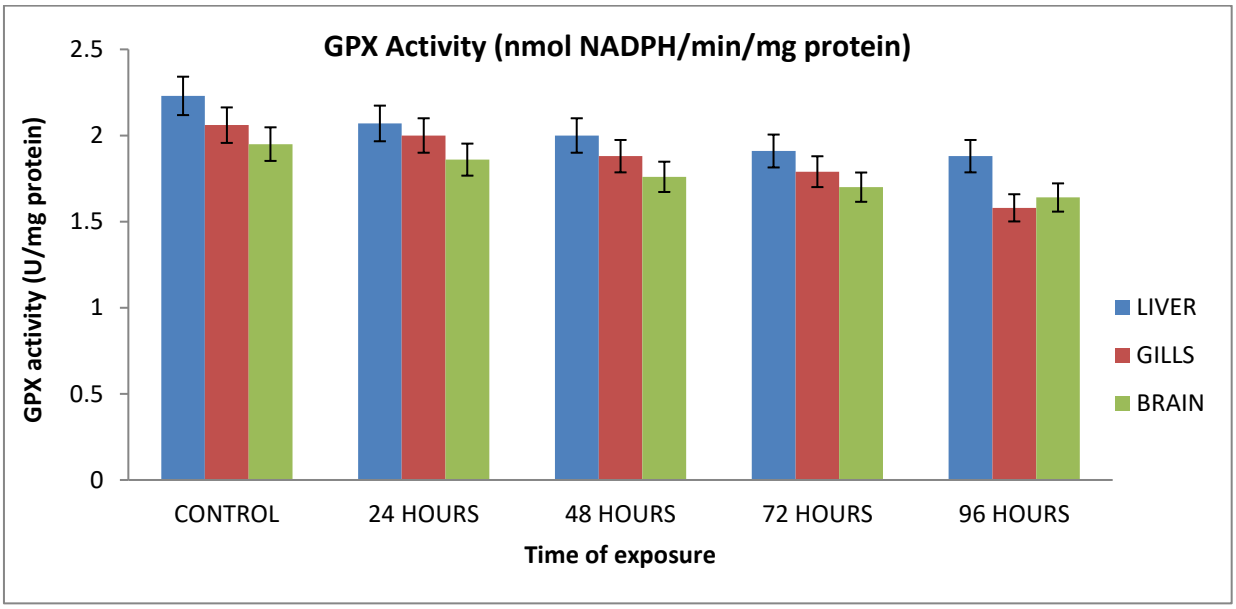

Figure 3: Effect of EQ on GPX Activity in liver, gill and brain of *Oreochromis mossambicus*

The activity of glutathione peroxidase was decreased in liver, gills and brain of *Oreochromis mossambicus* on EQ exposure at different time intervals. The decreased activity of Glutathione peroxidise may be due to deficiency of the antioxidant system [37].

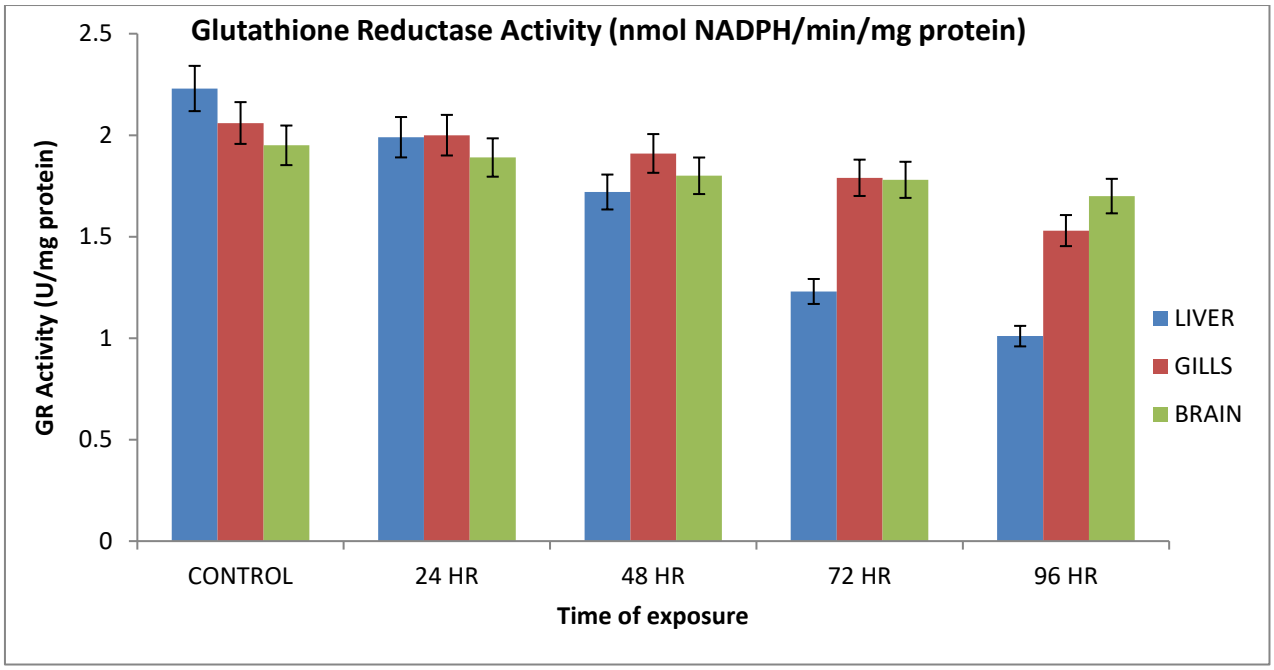

Figure 4: Effect of EQ on GR Activity in liver, gill and brain of *Oreochromis mossambicus*

The results showed that there was a significant decrease in GR activity on EQ exposure at different time intervals in all the three tissues. This decrease may be due to increase in oxidative stress which is an indication of impaired antioxidant defence mechanism as a result of excessive generation of free radicals. GR plays an important role in cellular antioxidant protection and adjustment processes of metabolic pathways. Decrease in GR activity could be due to a deficit in the production of GSSG (Glutathione disulphide) back from GSH (Reduced glutathione) mediated by Glutathione peroxidase.

SUMMARY AND CONCLUSION

The results revealed that the oxidative stress-response enzymes evaluated in this study displayed different responses to EQ. The present investigation indicated that EQ is toxic to fish and further substantiate earlier findings that antioxidant enzymes such as SOD, CAT, GR and GPX in fish could be effectively used as biomarkers of toxicity. The altered levels of antioxidant enzyme activities in the exposed fish can also be effectively used for better assessment of EQ toxicity in biomonitoring of aquatic environment.

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