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ORIGINAL ARTICLE **OPEN ACCESS**

Evaluation of oxidative stress markers in the liver of freshwater fish *Heteropneustes fossilis* exposed to an azo dye, Eriochrome Black T

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ABSTRACT
The objective of the current study is to evaluate the effects of the dye Eriochrome Black T (EBT) on the freshwater catfish species Heteropneustes fossilis. The antioxidant status and lipid peroxide index were used to assess the toxicity of EBT. Fish were exposed to 1 mg/l, 10 mg/l, and 20 mg/l concentrations of EBT for 96 hours. At the end of the experiment, liver tissue was sampled from control and exposed fishes for analysis of oxidative stress markers like glutathione reductase (GR), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and malondialdehyde (MDA) contents. The results revealed increase (P<0.05) in the activities of SOD, GPx and GR and a decline (P<0.05) in CAT activity, together with a concurrent rise in the levels of lipid peroxidation indicating oxidative damage caused by EBT in liver tissue. The liver antioxidant system of H. fossilis was most severely harmed by 20 mg/l EBT exposure. Our results suggest that EBT is hazardous to fish because it influences fish metabolism, leading to hepatocellular damage and liver dysfunction.
Keywords*: Eriochrome Black T; Heteropneustes fossilis; antioxidant enzymes; liver dysfunction*

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INTRODUCTION

Organic contaminants are among the most harmful toxicants because of their widespread dispersion in the surrounding environment [1]. The majority of them are resistant to deterioration in natural circumstances. Organic contaminants that enter an organism via water, food, and bottom sediments may accumulate in many tissues and organs, altering fish development, growth, metabolism, and reproduction [2,3]. There are many organic pollutants that degrade water quality and one of them is dyes, which are non-biodegradable soluble organic molecules [4,5].

The use of synthetic dyes as coloring agents or preservatives in the food, drug, cosmetic, and textile industries has expanded in recent times notably to enhance the sensory qualities and extend the shelf life of the finished products [6]. Among various synthetic dyes, the widespread use of azo dye is a result of their affordability, accessibility, and stability [7]. They have hazardous, mutagenic, and carcinogenic effects even at very low doses [8,9,10]. Therefore, the direct release of dye-containing industrial effluents into water bodies has been recognized as a major environmental issue in the twenty-first century [11,12,13]. Due to reports of azo dyes being found in the aquatic environment, the aquatic pollution brought on by dyes has increased along with the increase in the use of these colorants [14].

Apart from its role in metabolism, the liver is the primary organ for pollutants accumulation, biotransformation and detoxification [15,16]. Chemical exposure to contaminants can be confirmed using fish liver biomarkers [17]. Cellular responses to xenobiotics in aquatic organisms are represented by oxidative stress, which is defined as a disparity between pro-oxidant and antioxidant processes [18]. The uncontrolled production of reactive oxygen species (ROS) through oxidative stress can degrade DNA, lipids, and proteins that may results in cell damage, apoptosis, and necrosis [19,20]. To combat the detrimental effects of ROS, living organisms including fish acquired an antioxidant defense system comprised of different enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) [21].

Eriochrome back T (EBT) is one of the anionic, water-soluble azo dyes. It has numerous applications in the biological research field, the textile industry, and the resolution of water hardness [22,23]. It was discovered to be a water contaminant that seriously harms the ecosystem [13,24].

A comprehensive review of the literature showed very few studies on the effects of EBT upon fish [24,25]. Since fish is a source of high-quality protein for humans, it is vital to conduct investigations using fish as an experimental model to better understand the damage that azo dyes provide to aquatic biota, particularly teleosts.

This study is designed to examine the harmful impact of EBT on freshwater catfish, *Heteropneustes fossilis*, following a 96-hours of exposure. This species have a high economic value and are strong enough to adapt to challenging ecological circumstances Therefore, this fish can be considered as an ideal candidate to study the effects of EBT on the liver antioxidant defense parameters.

MATERIALS AND METHODS

Fish collection and acclimatization

The experimental adult fish were obtained from a local fish market in Aligarh and kept in plastic tubs containing nearly 50l of dechlorinated water for 4 weeks at 28°C ± 2 temperature and 12:12 hr photoperiod. Water was replaced daily before and after the fish were fed ad libitum.

Experimental design

Healthy fish were chosen and divided into control and experimental groups after 4 weeks of acclimatization. Experimental groups were subjected to 1 mg/l, 10 mg/l and 20 mg/l EBT exposure for 96 hrs. The experiment was performed in triplicate groups. All fish in the control and experimental groups were weighed and measured at the end of the exposure period to the nearest gramme (g) and centimeter (cm) respectively. Fish were subsequently sacrificed via ventral dissection in order to collect their livers.

Tissue collection

After being harvested, fish livers were cleaned of external blood, fat, and viscera of the tissues by washing them with chilled 0.6% normal saline. Tissues were then stored in deep freezer at -80°C for further analysis.

Homogenate preparation and enzyme analyses

Tissue homogenates were prepared in ice-cold 50 mM phosphate buffer saline (pH 7.0) by using a Potter-Elvehjem glass homogenizer to examine antioxidant enzyme activities. They were first centrifuged at 4°C for 10 minutes at 2500 rpm. A portion of the supernatant was collected for protein and lipid peroxidation, and the remainder was recentrifuged at 12000 rpm for 25 minutes at 4°C to yield the post mitochondrial supernatant (PMS). The PMS was kept at -20°C for further senzyme analysis.

Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) activities were determined using the methods of Das et al. [26], Aebi [27], Wood's [28] and Carlberg and Mannervik [29] repectively. The SOD activity was measured in unit/mg of protein, CAT in nanomole of H_2O_2/mg/sec, GPx in µmoles/min/mg protein and GR in µMoles NADPH oxidized/min/mg protein. The total protein content of liver was estimated at 660 nm according to the method of Lowry et al. [30] while lipid peroxidation (LPO) was determined by using the method of Ohkawa et al. [31] and expressed as nMoles TBARS/mg protein.

Statistical analysis

All the data is represented as mean ± standard deviation (SD). One-way ANOVA followed by DMRT (Duncan's Multiple Range Test) was performed to determine the significance of observed data among control and treated groups of fish by using SPSS version 26. Correlogram was plotted using minitab statistical software.

RESULTS

Lipid peroxidation (LPO)

A significant increase ($P<0.05$) in the liver LPO of EBT exposed *H. fossilis* was observed at 10 mg/l and 20 mg/l (except 1 mg/l) over the control group after 96 hrs of exposure (fig. 1). It can be seen that highest LPO was observed in 20 mg/l EBT treated group of the fish.

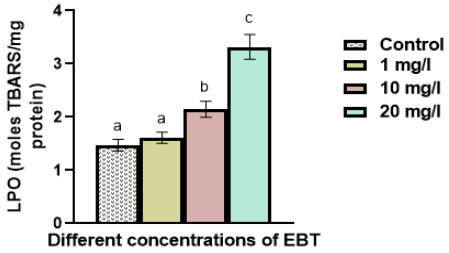

Figure 1: Effects of EBT on the liver lipid peroxidation (LPO) activity of *H. fossilis* after 96 hrs of exposure. Different superscripts represents the significant difference (P<0.05) among exposed groups and control

Response of antioxidant enzymes

SOD activity was observed to increased significantly (P<0.05) in the liver of EBT exposed groups of *H. fossilis* after 96 hrs of exposure compared to its control value while the highest SOD activity was noticed in 20 mg/l EBT exposed group (fig. 2). As shown in figure 2, significant decrease (P<0.05) in the CAT activity was noticed in EBT exposed groups compared to control one. However, 1 mg/l and 20 mg/l exposed groups did not show any significant difference between the CAT activities. GPx activity in the liver of *H. fossilis* revealed a significant concentration dependent increment (P<0.05) after EBT exposure (fig. 2). GR activity also increased significantly (P<0.05) in both 10 mg/l and 20 mg/l EBT exposed groups (except 1 mg/l) of *H. fossilis* over its control group (fig. 2).

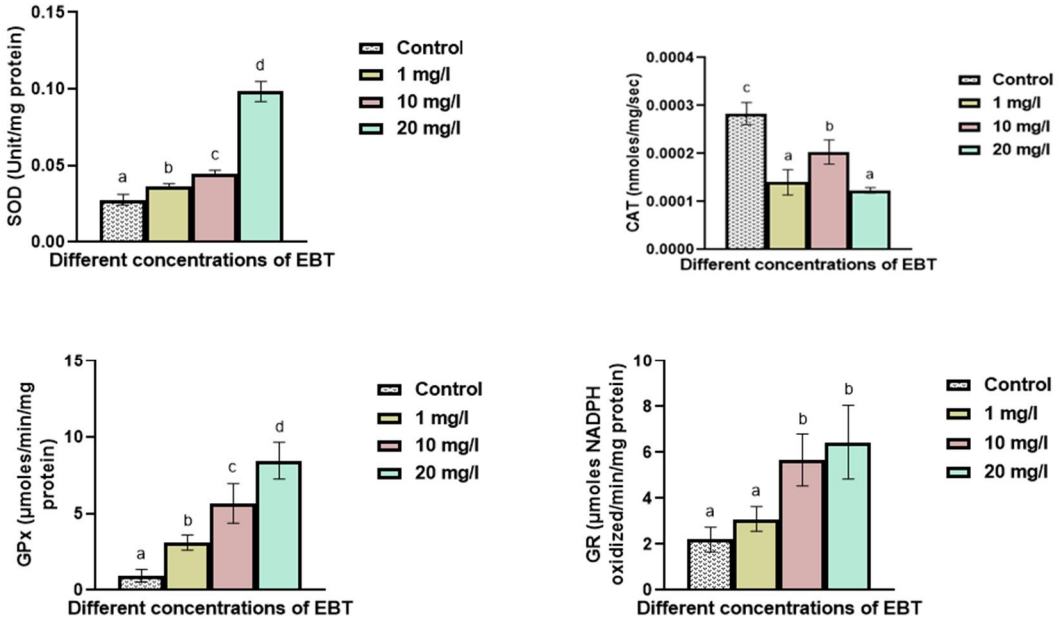

Figure 2: Response of different antioxidant enzymes after 96 hrs of EBT exposure in the liver of *H. fossilis*. Different superscripts represents the significant difference (P<0.05) among exposed groups and control

When LPO and antioxidant enzymes were compared among EBT-exposed groups, it was found that the strongest response of these biomarkers showed up at the highest dose of EBT (Table-1). CAT activity showed a similar reduction in both the 1 and 20 mg/l EBT exposed groups, although the response of GR is nearly identical in both the 10 mg/l and 20 mg/l EBT exposed groups, as shown in table-1.

Correlation matrix analysis

Correlation analysis of the selected parameters reveals a positive correlation among LPO, SOD, GPx and GR activities while CAT activity shows negative correlation with all these parameters (fig. 3). Correlogram chart shows a strong correlation among different parameters such as SOD which is positively correlated with both GR (0.82) and GPx (0.91). Similar to SOD, LPO also has positive correlation with both GPx (0.95) and GR (0.90). The strongest positive correlation was detected between SOD and LPO (0.99). However, strongest negative correlation was observed between CAT and GPx (-0.74).

Table 1: Alterations in Lipid Peroxidation (LPO) and different antioxidant enzyme activities of EBT-exposed *H. fossilis*

Parameter	% change in LPO and different enzyme activities of exposed fishes from the control			
	Control	1 mg/l	10 mg/l	20 mg/l
LPO (moles TBARS/mg protein)	1.471 ± 0.109[a]	1.613 ± 0.107[a] (+4.54%)	2.146 ± 0.152[b] (+51.22%)	3.318 ± 0.234[c] (+125.69%)
SOD (unit/mg protein)	0.028 ±0.003[a]	0.037 ± 0.002[b] (+23.79%)	0.045 ± 0.002[c] (+63.83%)	0.098 ± 0.007[d] (+221.78%)
CAT (nmoles/mg/sec)	0.00028	0.00014[a] (-42.59%)	0.0002 [b] (-33.94%)	0.00012[a] (-58.43%)
GPx (µmoles/min/mg protein)	0.944 ± 0.409[a]	3.116 ± 0.492[b] (+226.91%)	5.671 ± 1.299[c] (+801.06%)	8.461 ± 1.209[d] (+710.91%)
GR (µmoles NADPH oxidized/min/mg protein)	2.204 ± 0.545[a]	3.098 ± 0.536[a] (+47.59%)	5.672 ± 1.135[b] (+230.31%)	6.446 ± 1.612[b] (+283.91%)

Values are presented as means ± SD (n = 3). The different superscripts show significant differences (P <0.05)

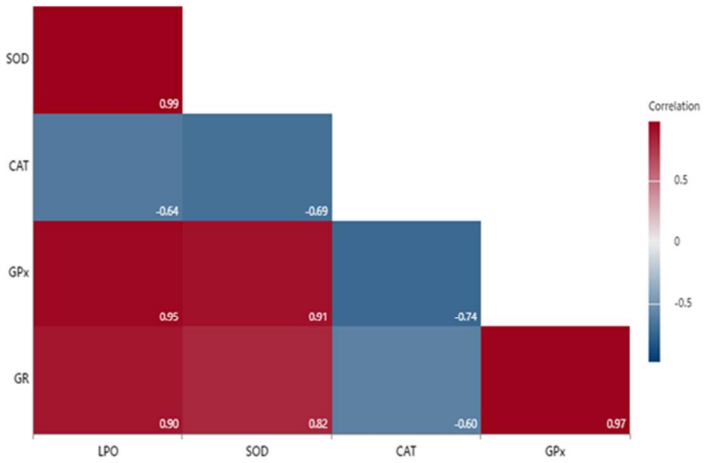

Figure 3: Correlogram of oxidative stress markers in the liver of *H. fossilis* after 96 hours of EBT exposure. The selected parameters, such as lipid peroxidation (LPO) and antioxidant enzyme activities such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) demonstrate a positive correlation among them with varying shades of maroon color whereas catalase (CAT) shows negative correlation with all the aforementioned parameters which is represented by the different shades of blue color.

DISCUSSION

Fish like other vertebrates, have antioxidant defense mechanisms as well as a variety of enzymatic and non-enzymatic components to mitigate the negative effects of ROS. Thus, the biochemical technique of assessing oxidative stress can be employed as a bioindicator to provide an early warning signs of stress in dye-exposed fish [32]. This study focused on the possible toxicity of EBT on the adaptive capabilities of *H. fossilis* liver in terms of antioxidant activity. The results revealed that the oxidative stress marker LPO and antioxidant enzymes examined in this study responded differently to this dye.

The higher LPO levels in exposed fish might have been caused by the generation of lipid hydroperoxides, which compromised plasma membrane integrity by interacting with membrane unsaturated fatty acid double bonds. *Anabas testudineus* and *Cyprinus carpio* treated with acid orange 7 and malachite green yielded similar results [32,33].

SOD transforms superoxide anions into H_2O_2, which is then broken down into molecular oxygen and water by CAT, whereas GPx catalyses glutathione mediated reduction of organic hydroperoxide and hydrogen peroxide that are produced in LPO [34].

SOD levels increased significantly in EBT-exposed groups of *H. fossilis*, indicating the onset of antioxidant defense in fish. Our findings are consistent with those of Sinha and Hanamghar [35], who discovered a similar rise in liver SOD levels in crystal violet-exposed *Labeo rohita*.

The decrease in liver CAT activity observed in dye-exposed *H. fossilis* after 96 hours of exposure could be due to enzyme depletion caused by stress, induced by increased H_2O_2 accumulation. Previous research has also found similar results after exposure to Red 195 dye and malachite green in liver CAT activity of *Oreochromis niloticus* and *Cyprinus carpio*, respectively [36,33].

There is a link between the actions of CAT and GPx in protecting organisms from ROS production. The significant increase in GPx activity in EBT-exposed *H. fossilis* demonstrates compensating role of GPx against ROS as CAT levels are reduced. In contrast to our findings, an increase in GPx was observed in the liver of *Labeo rohita* after crystal violet exposure [35].

A key component in shielding cells from oxidative stress is glutathione reductase (GR), which catalyzes the transformation of oxidized glutathione (GSSG) into its reduced form (GSH) [36]. After 96 hours of exposure to EBT, the results showed that *H. fossilis* had considerably increased hepatic GR activity at 10 and 20 mg/l doses of EBT which is an indication of the protective reaction of fish against EBT intoxication. An increase in hepatic GR activity was also found when *Oreochromis niloticus* and *Channa punctatus* were exposed to Red 195 dye and 2 amino benzene sulfonate, respectively [36,37].

After 96 hours of EBT exposure, the oxidative stress markers in the liver of *H. fossilis* were compared using correlogram which was plotted to determine the correlation among the selected parameters. The positive correlation among the parameters like LPO, SOD, GPx and GR represents their similar response to EBT intoxication whereas negative correlation of CAT with aforementioned parameters indicates the suppression of CAT activity in the exposed groups of *H. fossilis*.

CONCLUSION

According to the results of the current investigation, EBT was found to be hazardous to the test fish, *H. fossilis*. Its toxicity increased with exposure concentration. As a result, continuous exposure to this dye will almost certainly have a negative impact on the inhabitants of aquatic ecosystem and may eventually affect humans through the food chain. This study also suggests that changes in antioxidant enzymes and LPO could be used as sensitive indicators for assessing the detrimental effects of dyes and contributing in the development of discharge limitations. However, because the usage of azo dyes in industrial applications cannot be eliminated, improved techniques of disposal of the effluent must be developed. The existence of aquatic species in water bodies is seriously threatened if the current rate at which these dyes are introduced into water bodies is not regulated.

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CONFLICT OF INTEREST

The authors declared no conflicts of interest in relation to the work described in the present article.

AUTHOR'S CONTRIBUTION

Huma Naz carried out the experiments, evaluated the data, and wrote the manuscript. Dr. Huma Vaseem designed and organized the experiment, analyzed the findings, and proofread the manuscript.

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Ethics statement and informed consent - This does not apply.

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