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RESEARCH PAPER

Comparative study of endosulfan-effect on anti-oxidant enzymes (CAT and GPx) of air-breathing fresh water teleosts, *Clarias batrachus* (Linn.) and *Heteropneustes fossilis* (Bloch.)

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ABSTRACT

The fish, *Clarias batrachus*(Linn.) *and Heteropneustes fossilis* (Bloch.),were evaluated after 24 hrs of treatment of Endosulfan (5 ppb), on antioxidant enzyme (catalase and glutathione peroxidase). The sub-lethal concentration, 0.06 mg/liter and 0.05 mg/liter for 21 days treatment with endosulfan (95 % purity) for *Clarias batrachus* (Linn.) *and Heteropneustes fossilis* (Bloch.). Endosulfan exposure resulted into a significant induction increases ($p<0.05$-0.002) of GPx in all tissues studied. On the other hand, catalase activity was found to be considerably decreased ($p<0.01$-0.001) in all tissues. Results unequivocally confirmed peroxidative damage-dependent modulation of antioxidant level in liver, kidney and gill. Our study proposed, measurement of antioxidant level which may provide a useful bio-markers for aquatic-pollution monitoring and indicate that the activities of certain biomarkers in *Clarias batrachus* and *Heteropneustes fossilis,* on sensitive to pesticide.

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The polycyclic chlorinated hydrocarbon insecticide, Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano2,4,3-benzodioxathiepin 3-oxide), is a hazardous chemical (WHO, 1984). It is increasingly being used in India because of the ban on Endrin and the decline in the use of other organochlorine pesticides. Several instances of pollution of aquatic environment with Endosulfan residues have been reported. It is also known to be as one of the foremost pesticides found in water of major river and highly toxic to fish, in study conducted by CPCB, 2000(Central Pollution Control Board of India).

Pesticides may cause oxidative stress leading to a generation of free radicals and alterations in antioxidants or free oxygen radical scavenging enzyme systems. The antioxidant defense system has been progressively more studied because of the potential value of oxyradical mediated responses to provide bio-chemical bio-markers. The anti oxidant enzyme may induced by oxidative stress. The enzyme includes catalase, glutathione peroxidase and their reactions with oxy-radicals have been studied in fish. It is shown that the antioxidant of fish may be useful biomarkers of exposure to aquatic pollutants (Ahmad *et al.,* 2000). Knowledge of the major qualitative and quantitative similarities and differences in antioxidant defense systems among different species is necessary for the development of biomarkers .

MATERIALS AND METHODS

Fish:

Sexually mature *Clarias batrachus* (Linn.), length ranging from 40± 2-4 cm, weight 36±4-6 grams, and *Heteropneusties fossilis*(Bloch.), length ranging from 20± 2-3 cm with weight 25±5-6 grams, were collected locally and kept in large sand aquaria, each containing 50 liter water. Fishes were maintained by standard fish maintenance procedure (Elesceri *et al.,* 1998). Fish were acclimated 15 day prior to experiment. They were supplied daily with commercial fish feed at a rate of 2.5 % body weight and temperature was maintained at ambient laboratory temperature (30 ± 2.0 °C), pH 7.5-7.8, oxygen 7-8 mg/liter. Total hardness -134 mg/liter as $CaCo_3$, chlorides- 48 mg/liter, alkalinity –102 mg/liter. Fishes were transferred to a fresh volume of water every 24 hrs to minimize contamination from metabolic wastes of fishes. Feeding was stopped 24 hrs prior to experimentation.

Chemicals:

Glacial Acetic acid, Bovine Serum Albumin, Folin's Reagent, Na-K-Tartarate, $K_2Cr_2O_7$, DTNB, EDTA, , Na_2CO_3 , $CuSO_4$, $NaHPO_4$, Reduced Glutathione, were purchased from Sigma Chemicals, Co. USA and Merck , analytical graded reagents were prepared in double distilled water. Technical grade Endosulfan 96% pure obtained from, Parry Co. India, was used and stock

solution were prepared in acetone and diluted with water to prepare the desired concentration.

Experiment:

Endosulfan was dissolved in acetone solvent and a sub lethal concentration, of the pesticide was maintained in each water reservoir as reported (Mishra and Shukla, 1997 a). The equivalent volume (0.2 µL/L) of solvent was added to each control water tank. The sub-lethal and the median-lethal concentration of endosulfan for seven days were determined by employing the trimed Spearman-Karber method (Hamilton *et al.*, 1977). The fish were kept in two groups A- control exposed to solvent (acetone), and B-Exposure to Endosulfan for seven days. Antioxidant enzyme *viz.*, CAT, GPx, were estimated in liver, kidney and gill.

Biochemical estimation:

Post-mitochondrial supernatant preparation:

The specimens of *C. batrachus* and *H. fossilis* were sacrificed, the liver, kidney and gill, were quickly removed, cleaned and washed with fish saline. The tissue was homogenized in chilled phosphate buffer (0.1M in pH-7.4), containing KCl (1.17 %), using homogenizer. The supernatant was centrifuged at 10000 x g in Eltek Refrigerated Centrifuged (RC-4100) for 30 minutes at 4^0C to obtain supernatant, which was used for further biochemical analysis.

Antioxidant enzymes:

Catalase activity was assayed by the reference of Sinha, 1972. Method is based on the fact that dichromate/ acetic acid is reduced to chromic acetate in presence of H_2O_2 with formation of PCA (per chromic acid), blue, as un stable intermediate, chromic acetate thus produced (green), upon heating, is estimated calorimetrically at 580-610 nm.

Glutathione per-oxide assayed by the reference Butler *et al.,* 1963.The total amount of reduced GSH present in sample, which is indirectly related to the amount of Glutathione peroxide. Reduced GSH (having-SH groups) present in sample reduced the DTNB to stable yellow colour product, which can be measured calorimetrically at 412 nm.

Protein estimation:

Proteins from various samples were estimated by the method of Lowry *et. al.* (1951) using Folin's Reagent and BSA standard.

Statistical analysis:

The statistical analysis was done using student "t" test, was applied to determine significant ($p < 0.05$) differences between treated and control groups.

RESULTS AND DISCUSSION

Endosulfan, exposure resulted in significant increased in the activities of GPx ($p<0.006$-0.001), in liver, kidney and gill, when compared with control group. (Table 1 and Fig. 1 and 2), showing highest induction in gill. The induction of activity in gill was found to be 115.15 % when compared with corresponding control values. However, CAT activity decreased ($p<0.01$- 0.003), in all the tissues. The highest inhibition of CAT activity was found in liver (28.43%, when compared with control values.)

Free radicals play an important role in toxicity of pesticides and environmental chemicals. Pesticide chemical may induce oxidative stress leading to generation of free radicals and alteration in antioxidant or oxygen free radical scavenging enzyme system (Banerjee *et al.,* 1999). A single exposure to Endosulfan for 24 hrs elevated

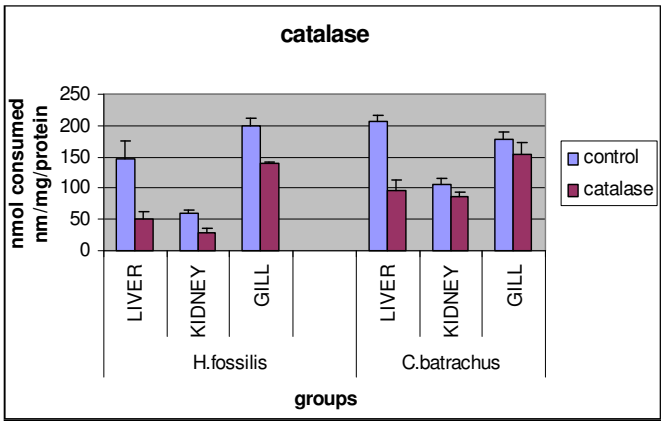

Fig. 1 : CAT activity in liver, kidney, gill of *H. fossilis* and *C. batrachus*

Fig. 2 : GPx activity in liver, kidney, gill of *H. fossilis* and *C. batrachus*

Table 1 : Effect on endosulfan on various biochemical parameters in fish tissues

Parameters		*Heteropneust fossilis* (Block)		*Clarias. Batrachus* (Linn.)	
		Control	Endosulfan exposed group# $	Control	Endosulfan exposed group# $
Liver	CAT*	146.09±30.11	49.59±13.18	206.80±10.09	96.91±16.17
	GPx**	98.69±12.72	154.10±11.23	191±8.09	288.13±17.58
Gill	CAT	198.93±11.98	138.92±3.75	177.38±12.50	153.07±19.88
	GPx	168.53±31.09	265±8.62	161.10±12.05	204.8±29.21
Kidney	CAT	60.08±12.88	28.44±7.50	104.68±20.17	86.42±18.15
	GPx	204.27±10.84	245.45±7.91	236.58±38.40	255±10.72

* CAT- nmol H_2O_2 consumed/min/mg protein, ** GPx- nmol NADPH oxidized/min/mg protein, # Single exposures of Endosulfan (5 ppb) are based on 24 h exposure, $ Values are expressed as mean ±SE (n=5);
Liver p<0.05, Gill p<0.05-0.002, Kidney p<0.01

Inhibition of CAT in liver
H. fossilis----- 33.93 %*
C. batrachus – 46.86 %
In kidney__
H. fossilis----- 46.77 %
C. batrachus – 82.41 %
In gill ____
H. fossilis----- 69.83 %
C. batrachus – 86.29 %
*lowest inhibition in liver of *H. fossilis*

Inhibition of GPx in liver
H. fossilis----- 156.14 %
C. batrachus – 150.85 %
In kidney__
H. fossilis----- 119.93 %
C. batrachus – 106.94 %**
In gill ____
H. fossilis----- 157.24 %
C. batrachus – 126.70 %
**lowest inhibition in kidney of *C. batrachus*

antioxidant enzymes level (CAT, GPx) in various fish tissues. GPx activity increases in all the tissues while; there was decrease in Catalase activity. MEP induces oxidative tissues damage to resulting from release of ROS (Hamcal *et al.,* 1995). Due to high reactivity of ROS most components of cellular structure and function may become potential target of oxidative damage, it may induce DNA damage as well as protein damage (Barmy *et al.,* 1996). As typical reaction during ROS- induced damage involves the peroxidation of unsaturated fatty acids (Kappus, 1987). To neutralize the impact of ROS, enzymatic antioxidants are activated. In Endosulfan exposure group of fish tissues, the levels of GPx and CAT were found elevated, apparently to provide protection against ROS damage. It has been seen that catalase activity was inhibited by free radicals, therefore, the activities of CAT were found significantly decreased in all the tissues. The inhibition of enzyme depends on the affinity to the inhibitor either to the enzyme or substrate co factor. These decreases in catalase activity could be due to the flux of ROS, which have been reported to inhibit CAT activity (Kono and Fridovich, 1982). The present results demonstrate that Endosulfan induces oxidative stress in fish tissues (liver, kidney and gill). Induction of antioxidants in response to Endosulfan exposure may prove to be a biomarker of aquatic pollution.

The present result suggests the following- (a) Endosulfan toxicity cause oxidative stress in fish studied due to generation of reactive oxygen species (b). To protect from deleterious effects of oxidative stress the fish produces enzymatic antioxidants. (c) Increased in antioxidants level may prove a useful biomarker of aquatic pollution.

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