
ENDOSULFAN INDUCED OXIDATIVE STRESS IN TILAPIA MOSSAMBICA**ANIL PATIL, REDDY, P.B**

Abstract: An array of contaminants provoke a variety of toxicity mechanisms including oxidative stress in living organisms. Endosulfan is one of the most unconvicted toxic pesticides and is responsible for a lot of pesticide poisoning incidents around the world. The present study is aimed to find out the effects of this pesticide on lipid peroxidation and anti oxidant enzyme activities in the liver a fresh water fish, *Tilapia mossambica*.

The exotic teleost fish *Tilapia mossambicus* of 15±1g was exposed to three different sub lethal concentrations of Thiodan 35 EC® for 24, 48, 72, 96 hours. The probit analysis showed that the LC₅₀ (lethal concentration) value of endosulfan (Thiodan 35 EC®) for 24, 48, 72 and 96 h were 0.9, 0.5, 0.1 and 0.05ppm for 96 hours. One third (30 µg/L) one tenth (5 µg/L) and one fifth (10 µg /L) of the LC₅₀ values were selected for sub lethal studies. Hepatic antioxidant enzyme activities like superoxide dismutase (SOD), catalase (CAT) and the level of glutathione (GSH) were measured to assess the oxidative stress (OS) status. Induction of the oxidative stress in fish exposed for 15 days to different sublethal concentrations of pesticide was evidenced by increased lipid peroxidation levels.

Results clearly shown that a significant increase (($P < 0.001$) in the activities of superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase, and glutathione reductase (GR) activities in the liver of fish from polluted site was observed. Overall, the outcome of this study reveal that alteration in the antioxidant enzymes, glutathione system and induction of lipid peroxidation reflects the toxic effects of pesticide which may cause oxidative stress in the *Tilapia mossambica*. The increased activity of antioxidant enzymes suggest that fish from polluted site were well protected from oxidative stress. Besides, the antioxidants responded positively in a concentration dependent pattern, thus, suggesting the employ of these antioxidants as potential biomarkers of toxicity associated with other pesticides and xenobiotics.

Keywords: Endosulfan, oxidative stress, lipid peroxidation, *Tilapia mossambica*,

Introduction: Anthropogenic impacts on aquatic ecosystems are prevalent in developed and developing countries. Over extraction of freshwater, mainly for industries and agriculture has lead to major deprivation of rivers, lakes and aquifers. Environmentally stimulated stresses often activate the endogenous production of reactive oxygen species (ROS), most of which are generated as derivatives of mitochondrial respiration. Therefore regular exposure to toxicants may enhance ROS-mediated oxidative damage. Pesticide pollution is an area of global concern due to their greater toxicity and persistence in the aquatic environment [1]. More amount of agricultural and industrial wastes enter aquatic environment and being taken up by aquatic organisms induce multiple changes. Some of them directly enhance ROS formation whereas others act indirectly [2]. Endosulfan is highly toxic and controversial agrichemical. It is an endocrine disruptor and can potentially accumulate in the tissues. Being highly toxic even EPA (1985) also kept this pesticide in category [3].

Oxygen is necessary for proficient energy production in all aerobic organisms. Reactive oxidative species (ROS) are formed as natural derivatives of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis [4]. But, due to environmental stress (UV, heavy metal and pesticide pollution, pathogens, heat exposure) ROS levels can

increase significantly and induce oxidative stress. Fish is a sensitive indicator of the quality of aquatic environment. They are adopted to live in diverse water surroundings but any unexpected change in water quality affects their physiology and metabolic activities. [5]. Liver is the first organ where pesticide is metabolized and detoxified.[6] Therefore, the present study is aimed to find out the impact of this toxic endosulfan on oxidative stress of liver in locally available fresh water fish, *Tilapia mossambica*.

Materials and Methods: Chemicals: The pesticide endosulfan Thiodan 35 EC® (Endosulfan 35%EC, Bayer Crop Science Ltd., Gujarat) has been used for the present study.

Fish sampling and acclimatization: Living healthy specimens of *Tilapia* ranging in weight 15±1g and in length from 8 cm to 10 cm of either sex or age were procured from local fresh water sources and acclimatized for lab conditions for 10 days. During this period, fish were fed every day with commercially available fish food twice a day (oil cake mixed with rice flour). The water in aquarium was changed daily.

Preparation of stock solution: A stock solution of commercial grade endosulfan was prepared using double distilled water. Successive dilutions of the stock solution were also prepared using earlier aerated and stored tap water. Each fish was weighed before and after the experiment and placed in its respective test chamber. After acclimation for 10 days,

healthy fish were selected from stock and transferred to another glass tank. Feeding was stopped one day before the commencement of the experiment.

Calculation of LC₅₀ value: Some fishes were used for the determination of LC₅₀ value and it was found to be 0.05 ppm for 96 hrs. Acclimatized fishes were divided into 4 groups and exposed to three sub lethal concentration of endosulfan with different sub lethal concentrations. One third¹/₃, one fifth¹/₅ and one tenth¹/₁₀ of the LC₅₀ values were selected for sub lethal studies. Group I control, Group II, Group III and Group IV were treated with 1/3, 1/5 and 1/10 respectively for 15 days. On 5th, 7th, 10th and 15th day five fishes were sacrificed by cervical decapitation. The liver was dissected out carefully, washed in ice-cold 1.15% KCl solution, blotted and weighed. They were then homogenized in homogenizing buffer (50mM Tris-HCl mixed with 1.15 KCL and pH adjusted to (7.4) using a motor-driven Teflon Potter-Elvehjem homogenizer. The resulting homogenate was centrifuged at 10,000 g for 20 min in a refrigerated centrifuge at 4 °C. The clear supernatants collected were used for protein estimation and assaying the activity of enzymes.

Biochemical Assays: Lipid peroxidation was assayed by measuring malondialdehyde (MDA) formation as described by Sharma and Krishnamurthy [7]. Superoxide dismutase (SOD) activity was determined by measuring the inhibition of autoxidation of adrenaline at pH 10.2 at 30°C as described by Misra and Fridovich [8]. Activity of catalase (CAT) was determined according the procedure of Clairborne [9] by following the absorbance of H₂O₂ at 240 nm, pH 7.0 and 25 °C. Activity of glutathione reductase (GR) was measured according to the method of Massey and Williams [10]. All assays were run in the triplicate. The protein content of the various fractions was estimated by the Folin-Phenol reaction method as described by Lowry *et al.* [11].

The data were expressed as mean ± S.D. Mean value for each group of fish was tested for significance by student's t-test to establish the validity of the findings.

RESULTS: Oxidative Stress Symptoms in Fish: Incidence of morphological abnormalities such as shedding of scales, discoloration, injury of skin, cracks and necrosis of fins, eye deformities,, lower lip extension and mucus secretion all over the body were observed in fish exposed with endosulfan. The proportion of these abnormalities was higher at higher concentration. The exposed fish also exhibited irregular, erratic and darting swimming movements and loss of equilibrium. After some time they tried to avoid the toxic water with fast swimming, jerking and jumping. Rapid opercula movement was observed as surfacing and gulping for air. At higher

concentrations of pesticide, the fish spin irregularly with strong jerks of the body. Their fins became hard and stretched due to stretching of body muscles. Finally, they stayed in vertical position for a few minutes with anterior side or terminal mouth up near the surface of the water and died.

Median Lethal Concentration (LC₅₀): The changes in lipid peroxidation (MDA), antioxidant enzymes SOD, CAT, GR in the liver of *Tilapia mossambica* exposed to three sublethal concentrations of endosulfan were presented in Tables (1,2,3,and 4). The result showed that the mortality rate increased with increasing concentration. From the results of mortality readings at 24, 48, 72 and 96 hr exposure LC₅₀ values and 95 % confidence limits for endosulfan were calculated to be 4.53 µg/L, 4.09µg/L, 3.74µg/L, and 2.592 µg/L, respectively. One third¹/₃, one fifth¹/₅ and one tenth¹/₁₀ of the LC₅₀ values were selected for sub lethal studies. A dose dependent increase and time dependent decrease were observed in mortality rate, such that as the exposure time increases from 12 to 96 h, the median concentration was reduced.

Lipid peroxidation: The results of endosulfan effects in different sublethal concentrations of on TBARS formation in the liver of *Tilapia* are presented in Table 1. Lipid peroxidation determined by the formation of TBARS is one of the commonly used markers of oxidative stress. Endosulfan significantly (P< 0.5) induced the soluble thiobarbituric acid reactive substances (TBARS) level consistently in all experimental groups in the liver of *Tilapia mossambica* in fish of all experimental groups. A significant effect of both concentrations and duration of exposure (p < 0.05) was observed in the specimen exposed to pesticide.

The lowest TBARS formation was observed on the first day of exposure to 1.188 nmol/mg protein and after that there was gradual non linear increase in TBARS formation in the liver tissue with the progression of the experiment, with the highest TBARS formation on the day 15 in the fish of Group IV.

Antioxidant Superoxide dismutase Enzyme Activity: The activities of the enzyme SOD in the liver of the fishes exposed to endosulfan are presented in Table 2. SOD activity was significantly (p < 0.05) elevated in the liver at all the concentrations of pesticide in the experimental group.

The elevated level of SOD activity is concentration and time dependent. The percentage of increase is 28% and 101% at the highest concentration on day 1 and 15 of the pesticide exposure in group III and IV respectively, as compared to control.

Catalase is a major antioxidant enzyme found in

almost all aerobic organisms. The activity of the enzyme differs significantly in different tissues, and elevated level in organs with high oxidative stress [12]. In the present study the activities of CAT did not follow the same pattern as SOD. Though CAT activity (table.3) in the liver was significantly different from the control ($p < 0.05$) and decreased from 79% on day 7 to 27% on day 15 following exposure to $1/3$ of LC_{50} value of endosulfan (Table 3). The elevated level catalase in treated fish may be due to higher biosynthetic and detoxifying activities of liver hence it needs extensive energy supply provided by oxidative metabolism.

As in CAT and SOD the glutathione reductase level was elevated ($p > 0.05$) in the liver of all experimental fish but less when compared to the SOD. It was also found that the increase was both concentration and duration dependent. GR activity increased significantly to 44% in the liver on day 10 to 73% on day 15 following exposure to $1/3$ of the pesticide (Table 4).

4. Discussion: Fish are cold blooded animals and are very sensitive to the surroundings. They often used as model organisms for ecotoxicological studies as they share a high amount of sequence and functional homology with mammals, including humans. They have also the ability to accumulate toxic substances and respond even to low concentration [13]. For that reason, the use of fish biomarkers as indices of the effects of pollution are of increasing importance and can permit early detection of aquatic environmental problems [14] [15] [16].

The results show that the toxicity of endosulfan for *Tilapia mossambica* is both time and concentration dependent. The result of the LC_{50} (median lethal concentration) for endosulfan in the present study at 96 h was 2.592 $\mu\text{g/L}$, The LC_{50} value obtained for *Tilapia* in this study is higher than that reported by previous studies [17] [18] [19]. Toxicity of chemicals to aquatic organisms has been shown to be affected by age, size and health of the species [20]. Physiological water quality amount and kind of aquatic vegetation, concentration chemical and its exposure also significantly influence such studies [21] [22].

The elevated level of lipid peroxidation in the liver of *Tilapia mossambica* in response to the exposure to endosulfan in the present investigation suggests that there is increased production of ROS. Increased ROS production may be associated with the metabolism of pesticide leading to the peroxidation of membrane lipids of the liver. The liver is renowned as place of detoxification, oxidative reactions and maximal free radical generation [23]. Previous investigations have reported the induction of LPO by pesticides such as deltamethrin [56], alachlor [24], Malathion [25] and butachlor [26] in fish. Lushchak *et al.* [27], however, did not record elevation of lipid peroxidation in the

brain and liver of goldfish *Crassius auratus* exposed to sublethal concentration of Roundup®. The different responses probably are functions of species, the time of exposure, type and concentration of stressors. The observed LPO resulting from ROS generated by the atrazine may lead to cell apoptosis. ROS and oxidative stress have been demonstrated to be triggers of apoptosis [28]. However, living organisms are prepared with mutually dependent flow of enzymes to relieve oxidative stress and repair damaged macromolecules, produced due to exposure to pollutants. In this cascade, SOD and CAT are the major enzymes in eliminating ROS formed during bioactivation of xenobiotics in the hepatic tissues [29] and the induction of SOD/CAT system provides a first line of defense against ROS. SOD help to dismutase superoxide radical O_2^- to hydrogen peroxide (H_2O_2). The increase in CAT activities in the liver as observed in the present study may be in response to H_2O_2 produced by SOD activity since CAT is responsible for the detoxification of H_2O_2 to water. Enhancement of CAT activity was observed in Cichlid fishes from polluted waters [30], in mullets [31], *Lepomis macrochirus* [32] and *Prochilobus lineatus* [33] exposed to herbicides. CAT activity, however, decreased after day 7 of exposure to atrazine, although the values obtained were significantly ($p < 0.05$) higher than control. Decrease in CAT activity after the 7th day of exposure could be due to decrease in the rate of reaction as a result of the excess production of H_2O_2 . In all, the increase in LPO, CAT and SOD activities in the liver tissue as reported in the present investigation supports the hypothesis that sublethal concentrations of endosulfan induced oxidative stress in *Tilapia mossambica* and could be an adaptive response to protect the fish from the pesticide -induced free radical toxicity. The elevated GR observed on day 10 and 15 could be part of the protective response in the tissue which suggest that GR pathways could efficiently be used to regulate the ROS formation during the contaminated period. This compensatory response accompanied with the induction of other antioxidants (SOD and CAT) evidently helps to prevent accumulation of free radicals and their products in stressed organisms. The result of these studies evidence that the biochemical responses are dependent on stressor type, species and exposure time. Furthermore, the pesticide may lead to the occurrence of transformation products in water with a potential or actual similar or higher toxicity than their parent [34]. Secondary metabolite products of endosulfan possibly could have affected the biochemical measurements in exposed *Tilapia*. Thus further studies are required to determine these biochemical changes after the exposure of fish to endosulfan and its transformation products.

5. Conclusions: Data from the present investigation suggest that assessment of antioxidant enzymes in fish could provide a valuable indicator of pollution of water bodies. The present investigation indicated that the endosulfan is toxic to fish and further authenticate earlier findings that antioxidant enzymes such as SOD, CAT and GR and LPO in fish

could be efficiently used as biomarkers of pesticide toxicity. This investigation could be useful in biomonitoring of aquatic environment, ecotoxicological researches in freshwaters as it provides data about antioxidant system response of fish exposed to various types of xenobiotics.

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Table. 1. Hepatic thiobarbituric acid reactive substances (TBARS) (nmol/mg protein) in *Tilapia* exposed to sublethal doses of endosulfan.

Conc. of Endosulfan of LC50 value	Exposure days			
	5	7	10	15
00	1.04 ± 0.04	1.03 ± 0.05	1.01 ± 0.07	1.00 ± 0.001
1/10	1.18 ± 0.05	1.25 ± 0.05	1.51 ± 0.14	2.41 ± 0.10
1/5	1.70 ± 0.19	1.84 ± 0.16	2.12 ± 0.11	2.54 ± 0.14
1/3	1.81 ± 0.11	2.02 ± 0.10	2.57 ± 0.11	2.61 ± 0.33

(Note: Data are presented as mean ± SE of five fishes in each group).

Table.2. Hepatic Superoxide dismutase (unit/mg protein) activity in *Tilapia mossambica* exposed to sub-lethal doses of endosulfan.

Conc. of Endosulfan of LC50 value	Exposure days			
	5	7	10	15
00	7.861 ± 0.09	7.882 ± 0.27	7.902 ± 0.11	7.952 ± 0.31
1/10	22.64 ± 0.43	28.61 ± 0.27	30.73 ± 0.47	33.80 ± 0.66
1/5	25.43 ± 0.48	24.80 ± 0.15	30.71 ± 0.41	33.82 ± 0.54
1/3	24.274 ± 0.54	30.853 ± 0.49	34.820 ± 0.53	34.91 ± 0.31

(Note: Data are presented as mean ± SE of five fishes in each group)

Table.3. Hepatic catalase activity (unit/mg protein) in *Tilapia mossambica* exposed to sub-lethal doses of endosulfan for 1-15 days.

Conc. of Endosulfan of LC50 value	Exposure days			
	5	7	10	15
00	10.32±0.11	11.13±0.2	10.32±0.01	12.03±0.3
1/10	11.32±0.13	12.13±0.21	13.32±0.2	14.03±0.13
1/5	12.72±0.12	13.43±0.31	14.32±0.2	14.13±0.10
1/3	13.42±0.13	14.03±0.40	15.02±0.5	14.93±0.33

(Note: Data are presented as mean ±SE of five fishes in each group)

Table 4. Hepatic glutathione reductase activity (nmol/mg protein) in *Tilapia mossambica* exposed to sub-lethal doses of endosulfan.

Conc. of Endosulfan of LC50 value	Exposure days			
	5	7	10	15
00	10.050 ± 0.04	10.211 ± 0.033	10.060 ± 0.15	10.052 ± 0.38
1/10	10.202 ± 0.51	10.401 ± 0.44	12.162 ± 0.61	12.911 ± 0.69
1/5	11.013 ± 0.60	11.544 ± 0.96	13.842 ± 0.55	15.524 ± 0.96
1/3	11.033 ± 0.64	12.023 ± 0.65	14.503 ± 1.1	17.402 ± 0.40

(Note: Data are presented as mean ± SE of 5 fishes in each group).

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