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# EFFECT OF ORGANOCHLORINE PESTICIDE, LINDANE ON THE ANTIOXIDANT ACTIVITY OF *OREOCHROMIS MOSSAMBICUS* (PETERS)

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**Abstract:** The impact of sub lethal acute toxicity of a synthetic organochlorine pesticide, Lindane on antioxidants activity of estuarine fish *Oreochromis mossambicus* was estimated. The median lethal concentration of lindane for 96 hrs was obtained as 0.82 ppm. In sub lethal treatments with the concentrations 1/5th, 1/10th& 1/15th of LC50 values (0.00546ppm, 0.0082ppm and 0.0164ppm respectively), antioxidants like superoxide dismutase and peroxidase increased whereas catalase activity decreased throughout the study period (7th and 14th day). Lindane, at its sub lethal concentration caused preternatural alterations on antioxidant activity in gill, hepatopancreas and muscle tissues of *O. mossambicus.* Observations of the enzyme activity showed that lindane has profound destructive effect on gill, hepatopancreas and muscle tissues. These alterations can be used to monitor the extent of pesticide contamination in the aquatic ecosystem. The modified activities of different antioxidant enzymes imply the activation of physiological mechanism to scavenge the reactive oxygen species produced by the toxicant exposure. The anti oxidant enzymes, superoxide dismutase, peroxidase and catalase can be used as biomarkers for non specific immune responses caused by acute exposure of pesticides or other similar aquatic pollutants. The study illustrated that lindane is harmful to *O. mossambicus* like aquatic organisms at sub lethal concentrations and application of pesticide close to bodies of water is dangerous threat to aquatic life.

***Key words:*** Lindane; Antioxidants; Catalase; Peroxidase; Dismutase; Pollution

## INTRODUCTION

Heavy use of organochlorine insecticides has led to the dispersal of these pollutants throughout the global environment. Hexachloro cyclohexanes (HCH) are one of the most commercialized organo chlorine insecticides. It has been classified in to two predominant products: technical HCH and purified gamma isomer, lindane [benzene hexachloride (BHC)]. The insecticidal properties of technical HCH were first described in the 1940s and the active gamma-isomer was named lindane after Van Linden, discoverer of the alpha and gamma-isomers. Lindane has a wide range of application in agricultural, medical and veterinary products as a very effective systemic insecticide (Martinez and Martinez-Conde, 1995; Hirthe et al., 2001; Golow and Odzi, 1994). Technical HCH contains about 60-70% alpha-HCH, 5-12% beta-HCH and 10-15% gamma-HCH. All HCH isomers are environmentally very significant because of their persistence and bioaccumulation. The beta isomer of HCH is the most persistent and bioaccumulative form and accounts for almost 90% of the HCH detected in human tissues and even breast milk (Solomon and Weiss, 2002). Lindane and other isomers of HCH can be transported over long distances by air currents (Shen *et al,* 2005). All HCH isomers vaporize and condense, touching down on oceans and freshwater bodies, where they may begin the cycle again. Lindane and other HCH isomers can bio-accumulate easily in the food chain due to its high lipid solubility and

can bio-concentrate rapidly in microorganisms, invertebrates, fish, birds and mammals, however, bio-transformation and elimination are relatively rapid when exposure is discontinued (WHO, 1991).

It is renitent to photolysis and hydrolysis with the exception of higher pH and degrades very slowly by microbial actions. Degradation takes place much faster under anaerobic conditions than in the presence of oxygen. These pesticides can reach natural waters either via transfer of the chemicals from contaminated soil or directly by spraying against target organisms (Oruç, 2010) and affects non-target organisms such as fish, prawn, mollusks and other aquatic organisms, which are of great economics importance to humans (Adhikari *et al.,* 1989). It induces convulsions and hyper excitability symptoms in mammals by attacking the central nervous system and act as a potent neurostimulator (Pimpao, 2007). Once liberated into the environment, lindane can distribute into all surrounding ecosystem. Lindane is stable in both freshwater and sea water. Like lindane, the alpha- and beta-HCH isomers are found in air, seawater, seabirds, fish, and mammals in the Arctic food web. The toxicology of alpha, beta- and gamma-HCH isomers has been studied extensively in mammals and to a lesser extent in fish and insects. The half life for environmental degradation of lindane, under humid or submerged conditions and field conditions varied from 30 days to 3 years (Ron van der Oost *et al.,* 2003).

India is the largest producer of pesticides in Asia and ranks 12th in the world and the pesticide lindane is widely used in agriculture (Duchiron, 2002). Evaluation of ecotoxicological risks caused by pesticides is based on the toxic effects to non-target organisms like fish (Suvetha *et al.,* 2010). Fish are used as excellent indicator of aquatic pollution due to their high sensitivity to environmental contaminants that may damage certain physiological and biochemical processes when contact with indicator of physiological and pathological changes (Orbea *et al.,* 2002). Moreover, biochemical and antioxidant parameters are used as health indicators to detect the structural and functional status of fish under stress condition (Ron van der Oost *et al.,* 2003; Morales *et al.,* 2004; Sa´nchez-Pe´reza *et al.,* 2005). Exposure to xenobiotics triggers the generation of reactive oxygen species (ROS). Aerobic organisms can generate superoxide anions, ($O_2$-), hydrogen peroxide ($H_2O_2$) and hydroxyl radicals (.OH) because of oxidative metabolism of xenobiotics. Lipids, proteins and nucleic acid are sensitive targets of ROS. Excessive ROS production leads to damage of cellular components and guides to oxidative stress. The cells have a complex defense system to protect themselves from ROS, including main antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and peroxidase and non-enzymatic scavengers (Pimpao, 2007). The primary defense is offered by enzymatic antioxidants, which have been shown to neutralize ROS. Fish tissues, specifically the liver and kidney are endowed with antioxidant defense system consisting of catalase (CAT), superoxide dismutase (SOD), peroxidase etc. to protect them from oxidative stress. Antioxidants contributes to the maintenance of relatively low level of the reactive and harmful hydroxide radical, the superoxide radical and hydrogen peroxide in the presence of $Cu^{2+}$ and/or $Fe^{3+}$. Such markers measured at the molecular or cellular level in fish have been proposed as sensitive "early warning" tool in environmental quality assessments (Suvetha *et al,* 2010).

## MATERIAL AND METHODS

### Collection and laboratory maintenance of test organism

Experimental fishes of almost similar size (8-10cm) have been collected from the Fisheries station, Kerala University of Fisheries and Ocean Studies, Puthuvyppu. The fishes were acclimated to laboratory condition for a week. A commercial diet with known proximate composition has

been given ad libitum. The Temperature in the tank during the experiment was maintained at 26 to 27°C, pH at 7 to 7.5, Dissolved oxygen 6 to 6.8 mg/L and salinity at 0 ppt. The saturation of oxygen was maintained by giving aeration in the tank. Healthy fishes were selected irrespective of the sex.

## Lindane Test Solution

Lindane (99% pure) has been obtained from Rajeev Gandhi Centre for Biotechnology (RGCB). Test solutions were prepared prior to the experiment. Since, lindane is insoluble in water; acetone is used as the solvent to prepare stock solution. The stock solution was prepared in order to get a solution of 1ppm when 1 mL of the stock was added to 1 L of double distilled water. Subsequent serial dilution was made from the stock solution.

### Determination of LC50

A static renewable bioassay method was adopted to determine the 96 hour LC50 (Sprague, 1969). After the period of acclimatization, six active and healthy specimen *Oreochromis mossambicus* were transferred into each of a number of aquaria containing 20 liters of aerated water with required concentrations of lindane. Two replicates for each concentration including control were kept for finding the lethal concentration. The fishes were unfed during the period of exposure and the number of live fishes was counted in every 24 hour. The concentration at which half of the fishes were killed is noted and confirmed by repeating the experiment. The sub lethal concentrations (1/5th, 1/10th and 1/15th of LC50) were selected according to the obtained LC50 value.

## Experimental Design

For conducting the biochemical study, *Oreochromis mossambicus* (15±2g) were taken in the three separate tanks that contain desired amount of toxin. The sub lethal test concentrations for acute study were selected as 0.00546ppm, 0.0082ppm and 0.0164ppm (1/5th, 1/10th & 1/15th of LC50) of lindane. Fishes in another separate tank were kept as control. The experimental animals were dosed for seven days. Daily the water in the tanks was replaced with water containing the same concentrations of toxin to avoid reduction in concentration by degradation. During the experimental period of 7 and 14 days the animals were fed with the same diet so as to avoid the effects of starvation on normal physiological processes and antioxidant stress. Any other factor likely to influence the toxicity was nullified by maintaining suitable control in tanks that contained no toxin.

After the experimental period the fish were killed by pithing (by damaging the brain and severing the spinal cord between the head and trunk region using a sharp needle) and the tissues viz gill, liver and muscle were removed from its body. They were washed in ice-cold 0.33M sucrose and blotted dry and the desired amounts of tissue were weighed and used. The soluble protein was estimated by using Lowry's method (Lowry *et al.,* 1951).

## Antioxidant activity

Superoxide dismutase activity was estimated by the method of Das *et al.* (2000). Peroxidase activities in tissues were estimated by the method of Addy and Goodman (1972) and catalase activity by the method of Sinha (1972).

## Statistical analysis

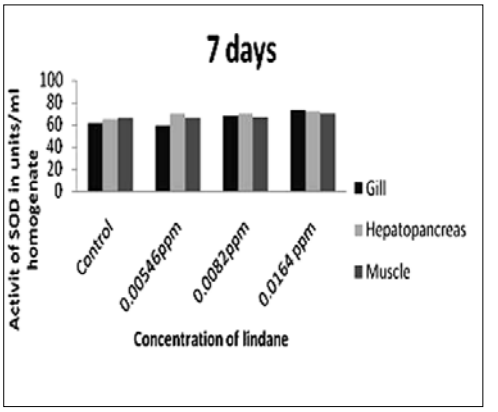
The probit mortality was found out using SPSS software 16.0 and biochemical data processed using SPSS 13 statistical program. All data were expressed as arithmetic mean ± SD, for the analysis of the experimental parameters Student's -t test was used.

## RESULTS

Probit Analysis confirms the LC50 value of lindane for *Oreochromis mossambicus* was estimated experimentally to be 0.082 mg/L. The tissues such as gill, hepatopancreas and muscle of *O. mossambicus* on exposure to sub lethal concentrations of lindane, (0.00546ppm,

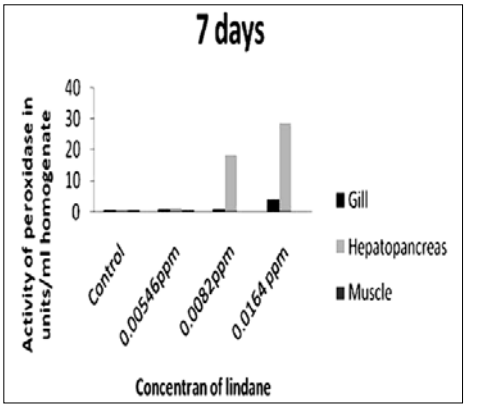
0.0082ppm and 0.0164ppm) for 7 and 14 days, were showed alteration in antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and peroxidase activity. The results showed that the activities of antioxidant enzyme, peroxidase ($p \geq 0.001$) (Table 2 and Fig.3 and 4) was strongly induced during almost all treatments whereas superoxide dismutase (SOD) does not show any significance at $p \geq 0.001$ (Table 1 and Figs.1, 2). The activities of CAT decreased significantly ($p \geq 0.001$) (Table 3; Fig. 5, 6) during chronic exposure of lindane.

Fig 1




**Figs 1 and 2.** Activity of SOD in tissues after 7 and 14 days exposure to sub lethal concentrations of lindane.

Fig 3




**Figs 3. and 4.** Activity of peroxidase in tissues after 7 and 14 days exposure to sub lethal concentrations of lindane.

Fig 2

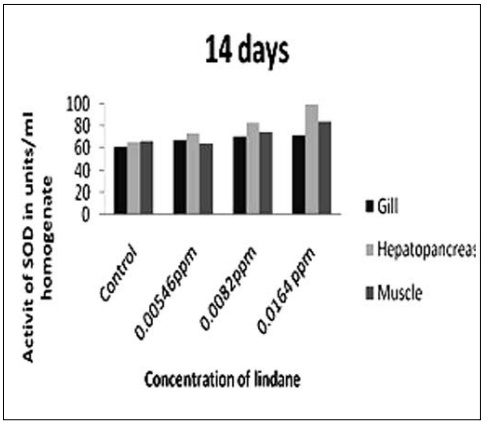



Fig 4

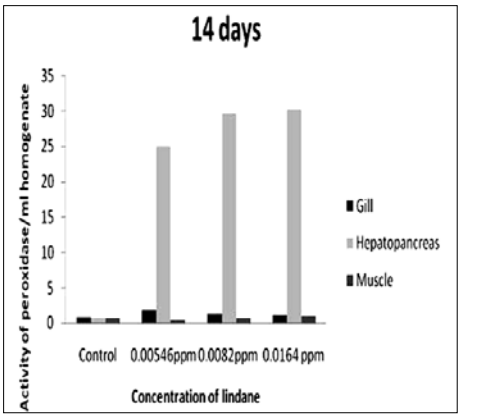


Fig 5

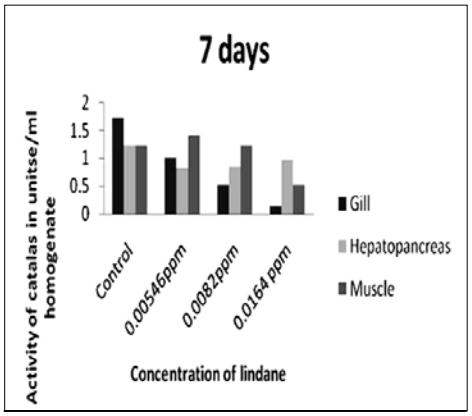
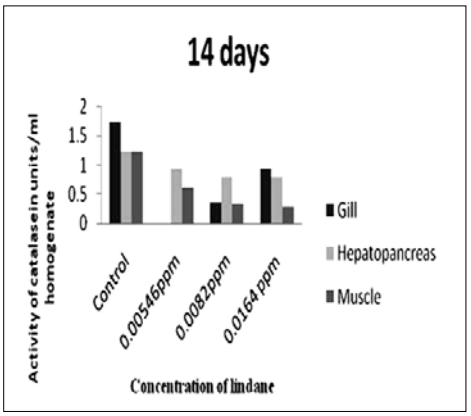



Fig 6




Figs. 5 and 6. Activity of catalase in tissues after 7 and 14 days exposure to sub lethal concentrations of lindane

Table 1. Effect of sub lethal concentration of Lindane in different tissues of *O. mossambicus* exposed for 7 and 14 days on the level SOD

| Days of Exposure | Tissue Analysed | Concentration of lindane | | | |
|---|---|---|---|---|---|
| | | Control | 0.00546ppm | 0.0082ppm | 0.0164ppm |
| 7 Days | Gill | 61.03±3.14 | 58.85±0.69 | 67.83±1.91 | 72.84±3.77 |
| | Hepatopancreas | 65.24±3.14 | 69.84±1.42 | 70.10±0.51 | 71.81±0.19 |
| | Muscle | 66.68±4.01 | 66.16±2.39 | 66.71±1.94 | 70.02±1.76 |
| 14 Days | Gill | 61.03±3.14 | 67.83±0.19 | 71.01±0.33 | 72.05±1.38 |
| | Hepatopancreas | 61.03±3.14 | 67.83±0.19 | 71.01±0.33 | 72.05±1.38 |
| | Muscle | 66.68±4.03 | 64.33±2.14 | 74.66±0.35 | 83.67±2.81 |

Value± SD* significant level at 0.001

Table 2. Effect of sub lethal concentration of Lindane in different tissues of O. mossambicus exposed peroxidase

| Days of Exposure | Tissue Analysed | Concentration of lindane | | | |
|---|---|---|---|---|---|
| | | Control | 0.00546ppm | 0.0082ppm | 0.0164ppm |
| 7 Days | Gill | 0.811±0.19 | 1.09±0.03 | 1.34±0.07 | 4.25±1.69** |
| | Hepatopancreas | 0.66±0.21 | 1.69±1.219* | 18.59±6.15* | 28.87±9.41* |
| | Muscle | 0.67±0.07 | 0.84±0.40 | 0.42±0.04 | 0.40±0.08 |
| 14 Days | Gill | 0.81±0.19 | 1.79±0.19 | 1.30±0.12 | 1.16±0.09 |
| | Hepatopancreas | 0.66±0.21 | 25.10±8.53* | 29.64±9.12* | 30.25±8.65 |
| | Muscle | 0.67±0.07 | 0.45±0.05* | 0.75±0.11* | 1.01±0.29 |

Value± SD* significant level at 0.001

**Table 3.** Effect of sub lethal concentration of Lindane in different tissues of O. mossambicus exposed for 7 and 14 days on the level catalase

| Days of Exposure | Tissue Analysed | Concentration of lindane | | | |
|---|---|---|---|---|---|
| | | Control | 0.00546ppm | 0.0082ppm | 0.0164ppm |
| 7 Days | Gill | 1.74±0.45 | 1.01±0.38 | 0.52±0.15 | 0.13±0.09* |
| | Hepatopancreas | 1.23±0.10 | 0.83±0.18 | 0.85±0.13 | 0.96±0.18 |
| | Muscle | 1.23±0.10 | 1.41±0.13 | 1.24±0.05 | 0.53±0.17* |
| 14 Days | Gill | 1.74±0.45 | 0.06±0.21 | 0.36±0.19* | 0.93±0.08 |
| | Hepatopancreas | 1.23±0.10 | 0.94±0.15 | 0.78±0.15 | 0.79±0.08 |
| | Muscle | 1.23±0.10 | 0.62±0.10 | 0.35±0.05 | 0.27±0.09* |

Value± SD* significant level at 0.001

## DISCUSSION

The present investigation showed that the 96 h LC50 value of lindane to *O.mossambicus* was found to be 0.82 ppm indicating that lindane is toxic to fish at a very minute concentration. The LC50 value obtained from acute toxicity study of lindane on *O. mossambicus* was similar to the findings of Addy and Godman (1972). Mac Donald (1994) studied the acute toxicity for different organisms and obtained the LC50 value for *Cyprinus carpio, Fundulus heteroclitus, Mugil cephalus and Perca fluviatilis* were 0.09 mg/L, 0.06 mg/L, 0.068 mg/L and 0.049 mg/L respectively. Hermens and Leeuwangh (1982) observed LC50 of 0.049 mg/L for guppy. The zebra fish larvae also shows a much higher (0.11 mg/L ) 96 hour LC50 value of lindane (Ensenbach and Nagel, 1990).

The protein contents of gill, hepatopancreas and muscle after exposure to lindane was studied. There was a significant decrease of protein level in the gill, hepatopancreas and muscle of lindane treated fishes. During lindane intoxication, the energy requirement goes up owing to the detoxification and tissue repair processes. This increased energy demand increases the metabolic rate as well as oxygen consumption. The decrease in protein level in liver and muscle tissues may be due to meet the elevated energy demands for metabolic purposes. Another hypothesis has been advanced to explain the reduced protein level in *B. guerini* exposed to pesticide endosulfan by Reddy *et al.* (1991). He suggested that physiological compensatory mechanisms are activated to either to provide intermediate for deriving energy through Kreb's cycle or to compensate for osmoregulatory problems by increasing the free amino acid level in blood; such mechanisms would have possibly operated in the test fishes exposed to lindane in the present.

Studies on the antioxidants such as superoxide dismutase, catalase and peroxidase activity in different tissues after lindane exposure, there was an increased SOD, and peroxidase activity and decreased catalase activity on lindane exposure after 7 and 14 days. Peroxidase along with catalase and SOD are considered as the key enzymes within the antioxidative defense mechanism, which directly determines the concentration of O2 and $H_2O_2$ (Dautrimepuits *et al.,* 2004). The increased activity of SOD and peroxidase under toxic condition may be for counteracting lipid peroxidation and removing toxic $H_2O_2$ or the organic hydroperoxide formed during lindane exposure. The results obtained in this study demonstrate that the sub lethal concentration of lindane can cause changes in biochemical responses and antioxidant activity in fish .This alteration may be potentially

destructive on the survivability and normal functioning of *Oreochromis mossambicus*. The stress created by sub lethal concentration of lindane leads to increased activity of antioxidant enzymes such as peroxidase and SOD. Peroxidase activity significantly increased ($p \geq 0.001$) in the present study, Similar studies also observed by Shalini Verma and Dubey (2003) due to metal toxicity. Peroxidase activity might be expected to reduce the level of ROS by metabolizing $H_2O_2$. This increase in antioxidant activity is to minimize the potential effect of ROS in cellular level.

Superoxide dismutase activity in *O. mossambicus* does not show any significance but showed increasing trend, it can be explained as a compensatory mechanism against pesticide intoxication. Such increase in the activity of SOD due to lindane exposure may be an adaptive response to decrease the severity of ROS effect. Organisms are able to adapt to some chronic situations of high exposure to ROS by raising the expression of antioxidant enzymes to defend against oxidative damage. There are many reports on activation of selected antioxidant enzymes (activity and/ or expression) during metabolically stressed states of organisms. This increased activity of antioxidant enzymes is considered as a critical phenomenon in the protection against post free radical damage known as "preparation for oxidative stress" (Halliwell and Gutteridge, 1999; Hermes-Lima and Tania Zenteno-Savin, 2002).

Catalase along with glutathione peroxidase and glutathione-S-transferase removes the $H_2O_2$ produced by dismutation of $O_2$. - (Superoxide radical) by superoxide dismutases (SODs) and the hydroperoxides produced by lipid peroxidation (Vázquez-Medina *et al.,* 2011). In our study catalase activity was decreased in *O. mossambicus*. Decreased catalase activity may be due to reduction in NADPH concentration pertaining to the higher energy need or immense generation of free radicals on chronic exposure to lindane. This indicates the potential effect of lindane on inhibition of antioxidant enzymes as a result of superoxide accumulation. Similar observation was found in *Clarius batrachus* exposed to pesticide, phosphemidon (Thomas and Murthy, 1978) and in the renal and hepatic tissues of *Heteropneustes fossillis* exposed to Endrin and Sevin respectively (Thomas and Murthy, 1978; Li *et al.,* 2007). Pesticide-induced inhibition of catalase activity has been reported on the exposure of endosulfan (Pandey *et al.,* 2001). Sayeed *et al.* (2003) pointed out that a drop in CAT activity could be explained by the flux of superoxide radicals due to the oxidative stress caused by the exposure of Deltamethrin in liver, kidney and gill tissues of Channa punctatus. Similar observation was found in exposure to metal intoxication in the *Fundulus heteroclitus* (killifish) exposed to cadmium (Pruell and Enngelhardt, 1980); in the liver of *Sarotherodon mossambicus* exposed to many metals (Sing and Sivalingam, 1982); and in different tissues of *C.batrachus* exposed to mercury (Sahana and Jana, 1985).

## CONCLUSION

The results of the present study indicate that lindane exposure during acute and sublethal treatment induces significant changes in the biochemical and antioxidant parameters of *Oreochromis mossambicus*. The alterations of these parameters may provide a early warning signals for the determination of acute and sublethal toxic level of pesticides and their effects in aquatic medium. The findings of the present study also provide a better understanding of the toxicological endpoint of aquatic pollutants and to ascertain a safer level of these chemicals in the aquatic environment and protection of aquatic habitats.

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