International Journal of Research (IJR)
e-ISSN: 2348-6848, p- ISSN: 2348-795X Volume 3, Issue 01, January 2016
Available at http://internationaljournalofresearch.org

Chlordecone-induced changes in muscular antioxidant system of cichlid fish, *Etroplus maculatus* (Bloch, 1795)

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ABSTRACT

Chlordecone, an organochlorine compound used widely as an agricultural insecticide, miticide and fungicide, was exposed at sub lethal concentration (3.5 µg/ L) to cichlid fish, Etroplus maculatus for 24, 72 and 96 h. The acute toxic effects of chlordecone were evaluated on the muscle antioxidant system of fish by maintaining respective control groups. Antioxidant enzymes such as superoxide dismutase, catalase and glutathione reductase and the levels of lipid peroxidation and hydrogen peroxide generation were assessed in the muscle tissue. There was a significant decrease in the activity of antioxidant enzymes with concomitant increase in hydrogen peroxide generation and lipid peroxidation in the muscle of treated animal than that of control groups. Muscle biomarker enzyme, alkaline phosphatase decreased in all treatment groups than that of control groups. It was therefore concluded that chlordecone exposure caused acute toxicity in fish that was revealed by the induction of oxidative stress in the muscle of Etroplus maculatus.

Keywords: Etroplus maculates; muscle; antioxidant enzymes; lipid peroxidation; alkaline phosphatase

1. INTRODUCTION:

Chlordecone is odorless and colorless chlorinated polycyclic ketone primarily used as an insecticide to control banana root borer, rust mites, wireworms in tobacco fields, grass mole cricket, slugs, snails and fire ants (ATSDR, 1995). It has been reported that chlordecone has a high potential for bioaccumulation in fish and other aquatic organisms (ATSDR, 1995). Chlordecone is a stable chemical and has been shown to have high resistance to physical and biological degradation where it remains in the environment indefinitely (Eroschenko and Osman, 1986). After rainy episodes, rain water wash out chlordecone from soil, to the surface waters where it becomes a source of contamination for aquatic organisms. As a result chlordecone cause harmful effects on aquatic life, particularly fish and finally to human health through fish food consumption. The median lethal concentration for chlordecone in catfish determined using static acute toxicity bioassay has been reported as 0.24 mg/ L (APHA *et al.*, 1975). In *Etroplus maculatus* LC_{50}-96h of chlordecone determined by probit analysis was 35 µg/ L (Asifa and Chitra, 2015).

It has been reported that chlordecone readily metabolized into chlordecone alcohol by a cytosolic aldo-keto reductase enzyme and eliminated from the body primarily through biliary excretion into feces (Molowa *et al.*, 1986). In ecotoxicology, fish have become an excellent toxicological model especially for the contaminants which are likely to exert their effects on aquatic ecosystems. Fish are likely exposed to toxicants through various routes such as waterborne, gills, derma and dietary (Sloman, 2007). In teleost fish, gill, liver, muscle and kidney are the tissues widely used to assess the ecological, toxicological and pathological studies. Fish muscle is considered as an important, valuable and recommended food in the human

nutrition due to low content of fat and high content of proteins and mineral substances as well as optimal ratio of unsaturated fatty acids with cardio-protective effect. On the other hand, fish muscle may be the depositary for different contaminants, which occur in the water ecosystem (Andreji *et al.*, 2012). In the present study the risk assessment of one of the environmental contaminants, chlordecone was evaluated in muscle antioxidant system of cichlid fish, *Etroplus maculatus*.

2. MATERIAL AND METHODS

The Cichlid fish, *Etroplus maculatus* weighing 7 ± 0.5 g and length 7 ± 1.5 cm were collected from local fish farm near Parappanangadi, Malappuram district, Kerala, India, Fishes were acclimatized to the laboratory conditions in cement tank prior to experiments and were maintained in well-aerated tank (40 L capacity), which was dechlorinated and sustained with good aeration.

Preliminary test were conducted by maintaining water temperature as 28 ± 2°C during the experiment, oxygen saturation of water was retained at 70 and 100 %, and pH 6.5 to 7.5 using standardized procedures as per APHA (1998).

Technical grade organochloride insecticide, chlordecone (Kepone, decachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]-pentalen-2-one, 99.9% purity) was obtained from Supelco, USA. Malondialdehyde, NADPH and glutathione oxidized were obtained from SISCO Research Laboratories, Mumbai, India. Thiobarbituric acid and pyrogallol were obtained from Himedia Laboratories, Mumbai, India. All other chemicals were of analytical grade and obtained from local commercial sources.

Animals were grouped into five with ten fish specimens in each group. Chlordecone was dissolved in 1% DMSO therefore it is used as a positive control in the experiment. Single dose with three durations were done in present study as follows:

Group I: Solvent-free control group

Group II: Positive control group (1% DMSO)

Group III: Treatment group - chlordecone at 3.5 µg/ L for 24 h

Group IV: Chlordecone at 3.5 µg/ L for 72 h

Group V: Chlordecone at 3.5 µg/ L for 96 h

The fish was caught very gently using a small dip net, one at a time with least disturbance and were decapitated. Muscle tissue were dissected and stored at 4ºC until the analyses were performed. A 1% (w/ v) homogenate of muscle tissue was prepared in ice-cold normal saline with the help of a motor-driven glass Teflon homogenizer on crushed ice for a minute. The homogenate was centrifuged at 8000 *g* for 15 min at 4°C to obtain the supernatant, which was then used for the biochemical analyses.

Protein was estimated by the method of Lowry *et al* (1951) with BSA as the standard. Activity of superoxide dismutase (Marklund and Marklund, 1974), catalase (Claiborne, 1985), glutathione reductase (Carlberg and Mannervik, 1985), level of hydrogen peroxide generation (Pick and Keisari, 1981), level of lipid peroxidation (Ohkawa *et al.*, 1979), activity of alkaline phosphatase (Bessey et al., 1946) were measured in crude homogenate.

Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range test using statistical package SPSS 19.0. Differences were considered to be significant at $p<0.05$ against control group. Data are presented as mean ± SD for ten animals per group. All biochemical estimations were carried out in duplicate.

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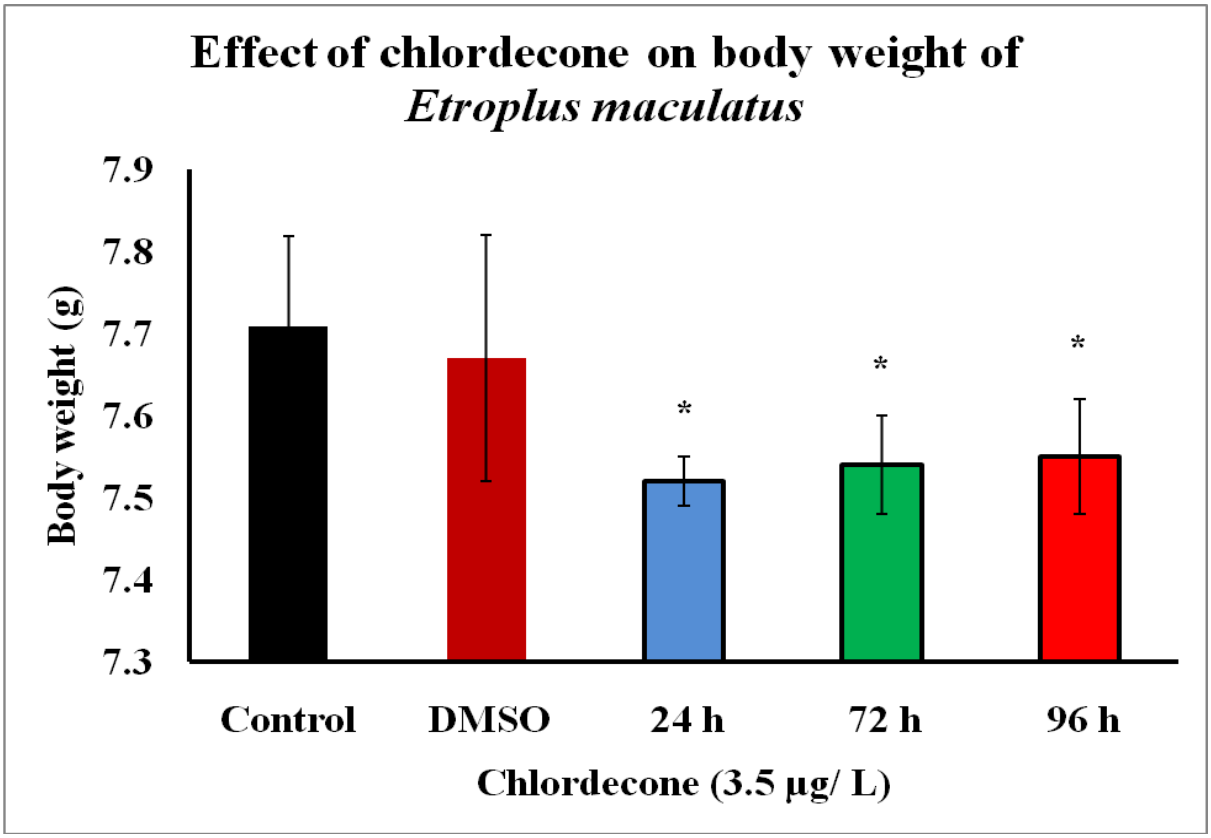
3. RESULTS AND DISCUSSION:

Water resources are indispensible part of human, at the same time threaten due to environmental contaminants originating from households, industries, agriculture and other anthropogenic activities cause harmful effects on biota. The contaminants that enter into the aquatic environment have been shown to alter the functioning of the aquatic ecosystem as a whole. Fishes are equipped with well developed antioxidant defense system. It is one of the important biochemical strategies that give protection to cells against deleterious effects of endogenous reactive oxygen species (ROS) by keeping their level relatively low (Paital and Chainy, 2010). Antioxidant defense system comprises of both non-enzymatic small antioxidant molecules and a cascade of antioxidant defense enzymes such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, (Halliwell and Gutteridge, 2007).

In the present study chlordecone-induced changes in the muscular antioxidant defense system was evaluated at sub lethal concentration (3.5 µg/ L) for 24, 72 and 96 h intervals in the fish, *Etroplus maculatus*. Some of the physiological stress conditions such as exposure to toxicants, nutrient or energy depletion due to anorexia or treatment related intake of food, sheer stress and hypoxia could cause oxidative stress in skeletal muscle (Clanton, 2007). Chlordecone showed significant decrease in the body weight of the animals when compared to control groups (Fig. 1) and this could be due to the systemic toxicity of the exposed compound.

Figure 1



Activities of superoxide dismutase (Fig. 2), catalase (Fig. 3), glutathione reductase (Fig. 4) decreased significantly in all treated groups when compared to the corresponding control groups. The levels of hydrogen peroxide (Fig. 5) and lipid peroxidation (Fig. 6) showed a significant increase in 24, 72 and 96 h of chlordecone treatment.

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Figure 2

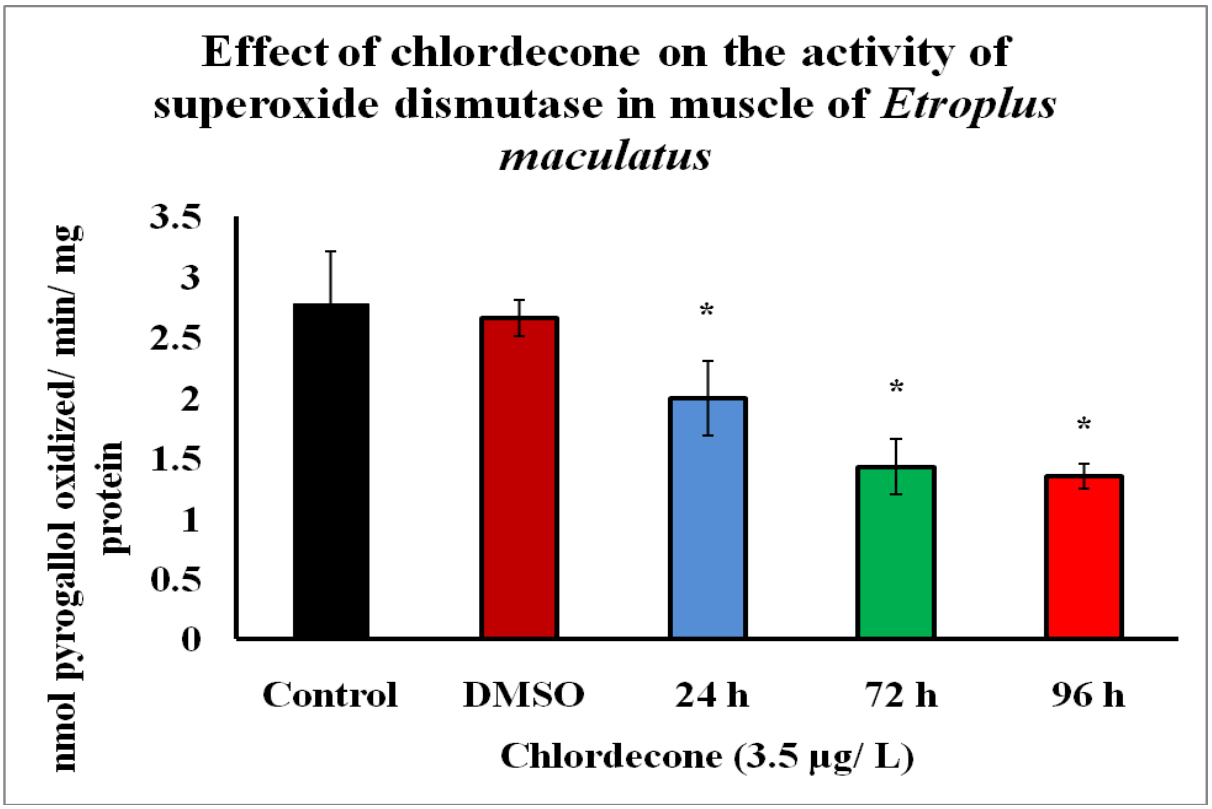

Figure 3

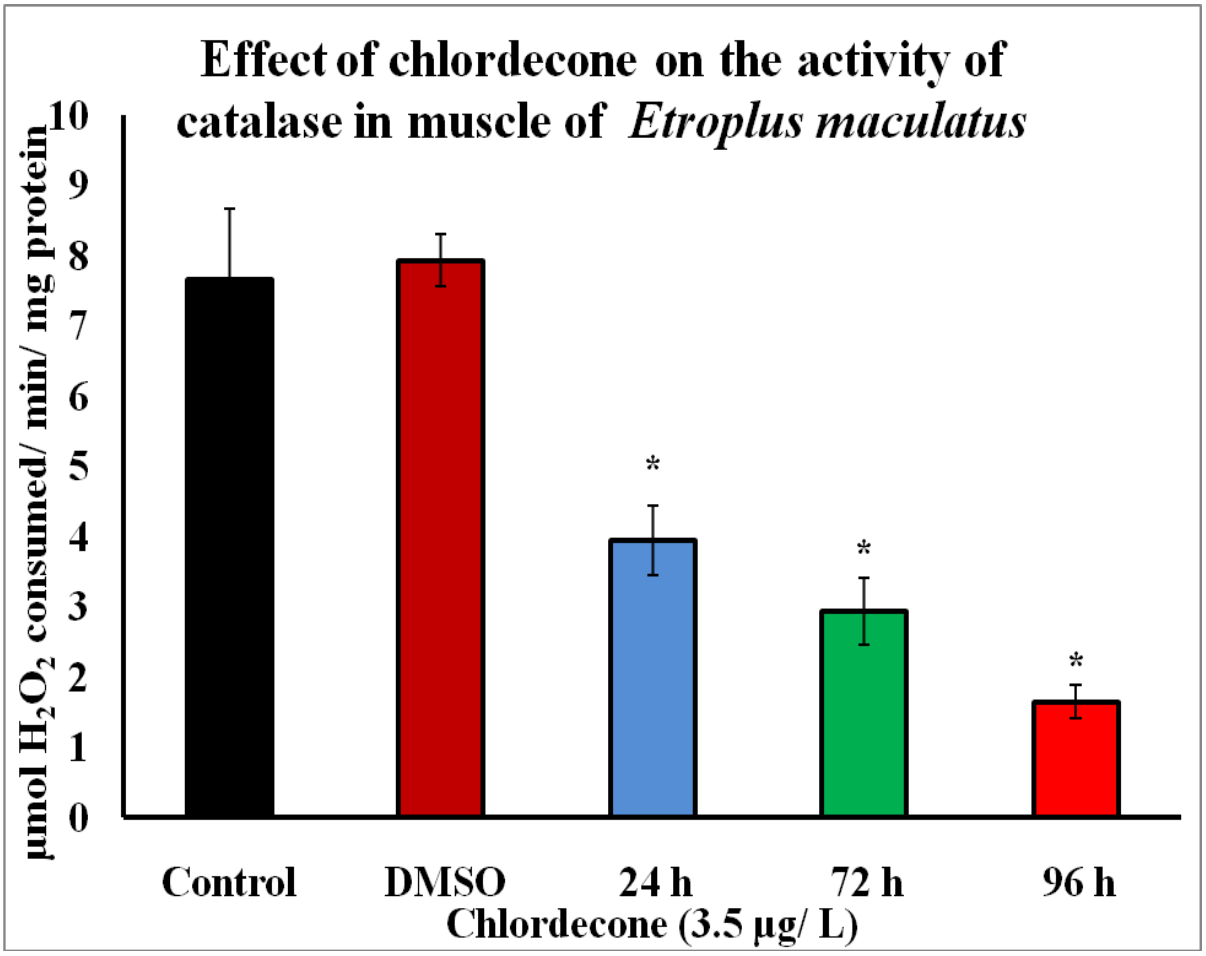

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Figure 4

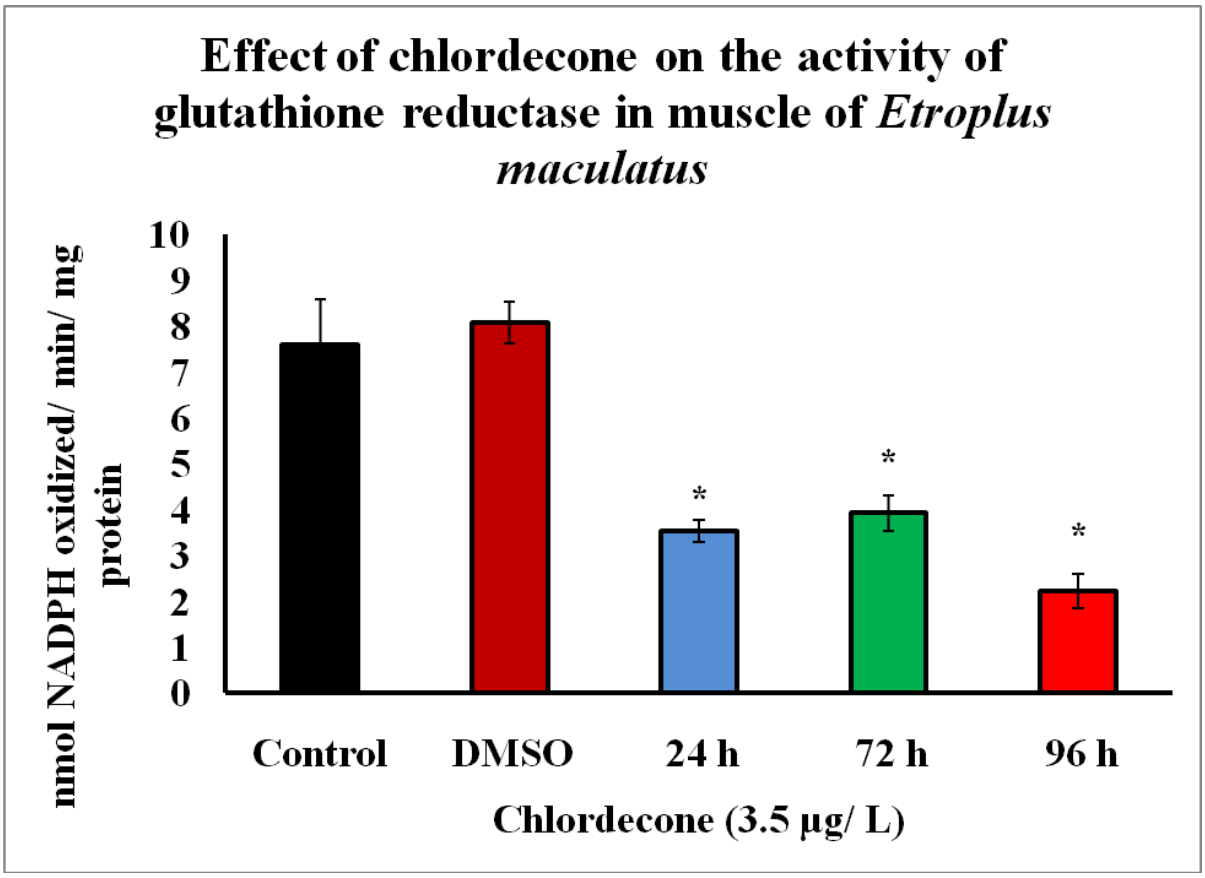


Figure 5

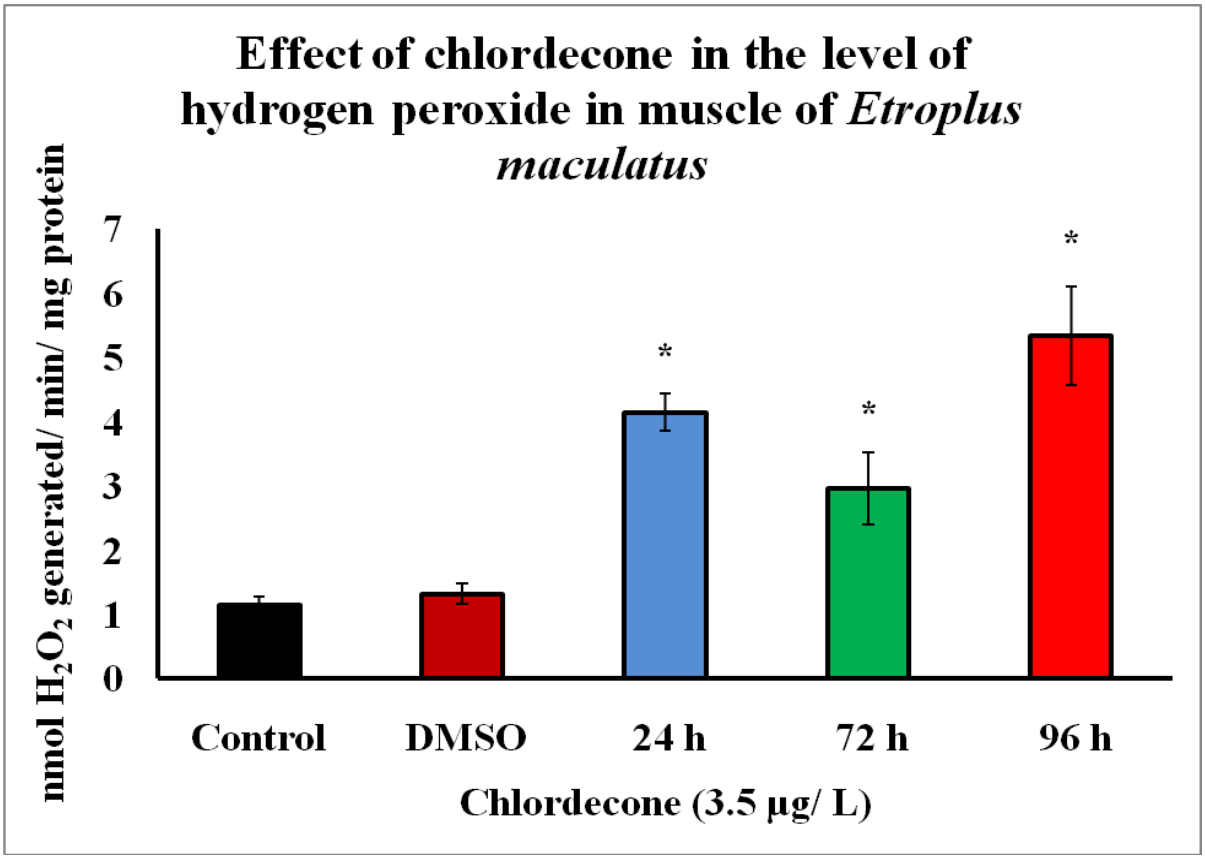
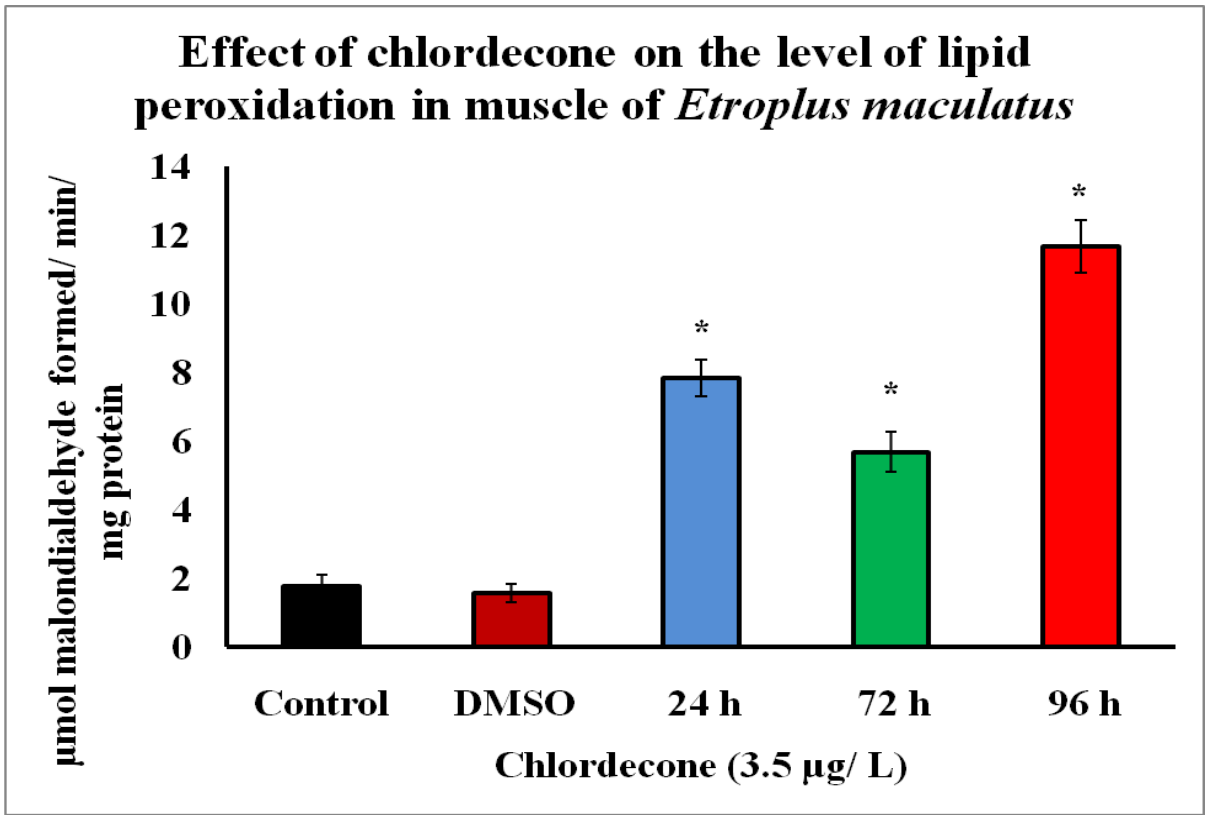


Figure 6



In general, superoxide dismutases prevent the accumulation of superoxide radical and it is rapidly eliminated. In the present study the reduction in the activity of superoxide dismutase reveals the failure of the enzyme to eliminate superoxide radicals. Accumulation of superoxide radical is therefore associated with oxidative stress in muscle tissues.

Hydrogen peroxide (H_2O_2) generated from superoxide is produced by mitochondria and NADPH oxidases and it is further reduced to water by the enzymes catalase and glutathione reductase/ peroxidase system (Brand, 2010). Catalase exists as a tetramer composed of four identical monomers, each of which contains a heme group at the active site. A significant decrease in the activity of catalase and glutathione reductase in all treatment groups of chlordecone that H_2O_2 generated is not eliminated and it was confirmed in the present study by a significant increase in the level of hydrogen peroxide in the muscle tissue.

H_2O_2 is one of the major free radical in the cell and thus leads to the induction of lipid peroxidation which disrupts the membrane lipid bilayer arrangement that may inactivate membrane-bound receptors and enzymes and increase tissue permeability (Girotti, 1985) as revealed by the increase in malondialdehyde level in the present study. Oxidative stress also leads to the fragmentation of peptide chain, alteration of electrical charge of proteins, cross-linking of proteins and oxidation of specific amino acids, and therefore lead to increased susceptibility to proteolysis by degradation by specific proteases in muscle tissue (Kelly and Mudway, 2003).

One of the marker enzymes, alkaline phosphatase decreased significantly in time-dependent manner when compared to corresponding control groups (Fig 7).

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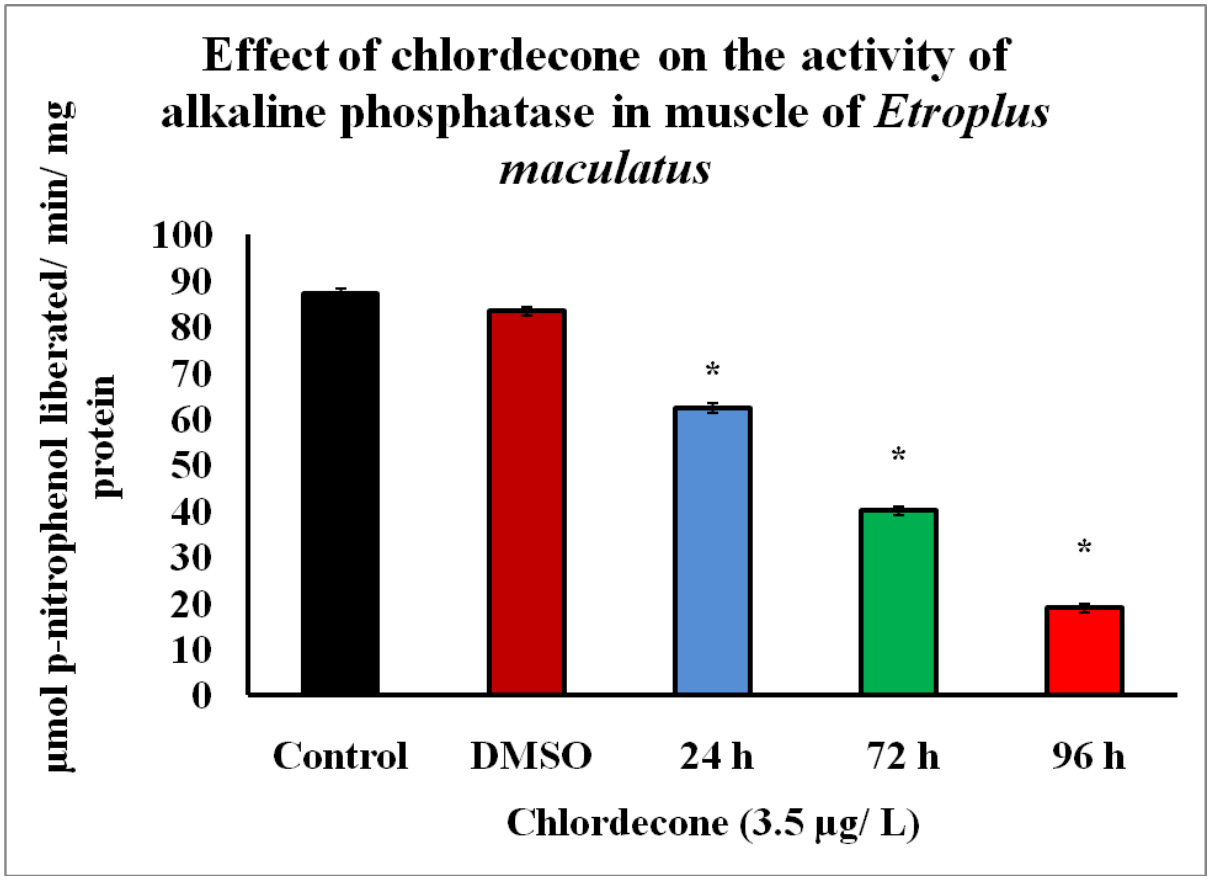
Fig. 7



Alkaline phosphatase are one of the stress marker enzyme and widely used as a diagnostic tool to assess the toxicity stress of chemicals in the living organisms (Harper, 1991). Alkaline phosphatase is a hydrolytic lysosomal enzyme and is released by the lysosomes for the hydrolysis of foreign material and it is also involved in the mediation of membrane transport and transphosphorylation. In the present study a significant decrease in the activity of alkaline phosphatase at all treatment groups indicate the decreased state of inter and intracellular membrane transport. This could be possibly due to the acute toxicity effect of chlordecone.

Therefore, the present study evaluates the toxic effect of chlordecone and was proved by the induction of oxidative stress and imbalance in the antioxidant defense system in muscle of the cichlid fish *Etroplus maculatus*.

4. CONCLUSION

The present findings consequently prove that the antioxidant enzymes may be highly recommended as useful early bio-indicator of environmental pollution by chlordecone in the aquatic ecosystem.

Acknowledgments:

Authors gratefully acknowledge the financial grant from Kerala State Council for Science, Technology and Environment (KSCSTE), Thiruvananthapuram, Kerala to carry out this study.

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