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Full Length Research Paper

Study on the changes in the levels of membrane-bound ATPases activity and some mineral status in λ cyhalothrin-induced hepatotoxicity in fresh water tilapia (*Oreochromis Mossambicus*)

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An attempt has been made to determine the deleterious effects of λ cyhalothrin- induced in fresh water *tilapia* (*Oreochromis mossambicus*) with respect to changes in the activities of membrane-bound ATPases (Na^+/K^+, Mg^+ and Ca^{2+} ATPase) and mineral status (sodium, potassium and calcium) in the liver. Significant ($p<0.05$) decline in the activities of membrane-bound ATPases and mineral status was observed in λ cyhalothrin-intoxicated fishes as compared to controls. A concomitant ($P<0.05$) elevation in the level of lipid peroxidation in liver was also observed. The results of the present investigation have indicated that the toxic effect of λ cyhalothrin-induced in fresh water tilapia (*Oreochromis mossambicus*) might be ascribable to its membrane destabilizing property or peroxidative action. Histopathological studies also proved its toxic potential in terms of the damage induced at organ level.

Key words: Histopathology, liver, λ cyhalothrin, membrane-bound ATPases, mineral status, *Oreochromis Mossambicus.*

INTRODUCTION

Pesticides are chemicals manufactured specifically to be toxic to living species and are released deliberately into the envioronment. The pesticide run offssociated with agricultural operations adjacent to estuaries and coastal environments may compromise the health of marine organisms (Bocquene et al., 1997). The homeostatic systems of fish are continuously challenged or stressed by the normal demands of the aquatic environment (Wedemeyer et al., 1984). Stress has been defined in physiological terms as a diversion of metabolic energy from animals normal activities (Barton and Schreck, 1987).

λ Cyhalothrin (chloro trifluoropropenyl or dimethylcyclopropane carboxylate) (Figure 1) is a pyrethroid insecticide used to control insect pests in a wide range of crops. λ -cyhalothrin was first approved for use in the UK in 1988 (Advisory Committee on Pesticides, 1988). The use of organochlorine pesticide has declined; the use of synthetic pyrethroid has increased with time and they represent the most popular synthetic insecticides. The use of synthetic pyrethroid in India has increased by 42% during last 5 years. Various studies have demonstrated that synthetic pyrethroids are more efficient in controlling insect pest menace and have a favorable persistence profile than conventional insecticides.

Pyrethroids are a group of man made pesticides similar to the natural pesticide pyrethrum, which is produced by chrysanthemum flowers. Synthetic pyrethroids are more stable in light and have higher insecticidal activity than products made from chrysanthemum flowers. Because of this efficacy, only small amounts of pyrethroids need be applied to control pests. Pyrethroids are toxic to fish and other aquatic organisms (Tomlin, 1994). Pyrethrins and pyrethroids act as contact poisons, affecting the insect's nervous system (Klassen et al., 1996). Insecticides are substances that prevent, destroy and kill insects.

Tilapias principally (*Oreochromis* species) are of major

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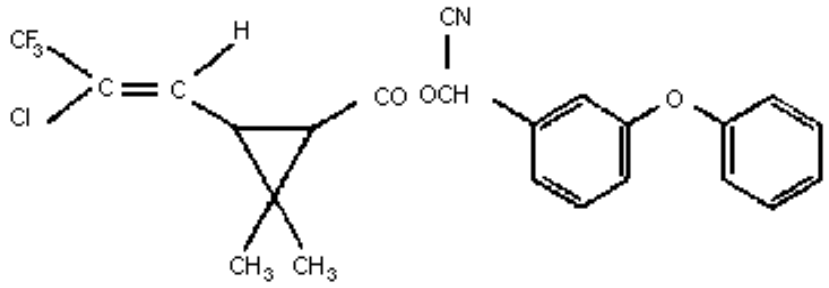

Figure 1. Chemical structure of λ Cyhalothrin.

importance to aquaculture in tropical and subtropical regions, contributing nearly a million tones to world aquaculture production in 1997 (FAO, 1999). The tilapias are important food fishes in tropical countries. The tilapias are characterized by a general tolerance to a wide range of environmental conditions including salinity (Prunet and Bornancin, 1989). Exposure to λ cyhalothrin on metabolic and morphologic aberrations in the liver tissue of the fishes is not yet explored. In the present study, we investigated the deleterious effects of λ cyhalothrin-induced hepatotoxocity in fresh water tilapia (*Oreochromis Mossambicus*) with respect to changes in the levels of membrane-bound ATPase activity, mineral status and lipid peroxidation.

MATERIALS AND METHODS

Chemicals

Epinephrine, tetraethoxy propane and λ cyhalothrin were obtained from M/s. Sigma Chemical Company, St. Louis. MO, USA. All the other chemicals used were of analytical grade.

Animals

Tilapia (*O. mossambicus*) of length ranging between 9 to 13 cm and weight 2 to 7 g collected from Pallathuruthy pond, Cochin, India were selected for the study. The fishes were kept in fibre plastic tanks and maintained at normal room temperature (30 ± 2°C, 12 h light/ dark cycle).

Experimental protocol

After acclimatization, the fishes were divided into four groups of 10 fishes each. Group I served as control. Group II were normal fishes exposed to acetone alone (vehicle control). Groups III and IV fishes were exposed to λ cyhalothrin [0.3 µg (dissolved in acetone)/L] and [1.1 µg (dissolved in acetone)/L] respectively, for the induction of hepatotoxicity. The tanks were covered with nylon nets. The toxicant solution was renewed every 24 h and the experiment was continued for a period of 15 days. At the end of the experiment, the fishes were killed and liver tissue excised was used for various biochemical analyses.

Biochemical assays

Levels of minerals in liver tissue were estimated according to the method of the AOAC (1980) using Atomic Absorption Spectrophotometer. Activities of membrane-bound ATPase activity were also determined in liver tissue. Mg^{2+} ATPase activity was determined by the method of Ohinishi et al. (1982). Na^+/K^+ dependent ATPase activity was determined by the method of Bonting *(*1970). Ca^{2+} ATPase activity was determined by the method of Hjerten and Pan *(*1983). Lipid peroxidation was determined by the method of Ohkawa et al. (1979).

Histopathology of λ cyhalothrin, a synthetic pyrethroid pesticide on the liver tissue in fish tilapia (*O. Mossambicus*) one of the most salinity tolerant species was determined by light microscopy. Slices of the liver tissues were fixed in 10% neutral buffered formaldehyde. Fixing prevent autolysis and putrification of tissues. Then it was dipped in different concentration of alcohol in ascending order and finally in absolute alcohol (10 min each) for removing water. It was kept in methyl benzoate until it sank and dipped in benzene for removing alcohol. The tissues were then infiltered with molten paraffin (60 to 70°C) for 1 h and 15 min. A boat was made filled with molten paraffin and the tissues were placed in it. The paraffin was then cooled until it hardened, enclosing the tissues.

Using a rotary hand microtome, sections of 4 to 5 µ thick paraffin infiltered tissues were made. The tissues were de-paraffinised with xylene and treated with 100, 90 and 70% alcohol (10 min each) for removing undesirable pigment and other materials. The sections were then stained with haematoxylin and counter stained with eosin and dehydrated with 70 and 100% alcohol for 10 min each. The sections were mounted using dibutylpthalate in xylene and examined under microscope.

Statistical analysis

All data were analyzed using ANOVA with the aid of SPSS 10.0 for windows. Data obtained were expressed as mean± SD. Multiple comparisons of the means were separated using the Duncan multiple range test at 5% probability.

RESULTS AND DISCUSSION

A significant ($P<0.05$) elevation was observed in the level of Ca^{2+} ions in group IV fishes (1.1 µg/L) compared with group I (control fishes) (Table 1). This is in accordance with an earlier reported study (Namikawa et al., 1992). Ca^{2+} is the key ion for normal activity of many enzymes (Hamet, 1995). Λ Cyhalothrin induced hepatotoxicity has been reported to enhance cyclase activity, resulting in increased formation of cAMP (Subash et al., 1978). cAMP phosphorylates several sites on the C-terminal

Table 1. Levels of Na^+, K^+, and Ca^{2+} in liver tissue (mg/g wet tissue) of normal and experimental groups of fishes.

	Control (Group1)	Acetone (Group II)	Pesticide+Acetone (0.3 µg/L) (Group III)	Pesticide+Acetone (1.1 µg/L) (Group IV)
Na^+	175.5 ± 12.62a	168.5 ± 12.13a	51.0 ± 10.87b	97.5± 7.02c
K^+	67.5 ± 4.86a	71 ± 5.11a,b	77.5 ± 5.58b	109 ± 7.84c
Ca^{2+}	38.8 ± 2.83a	41.55 ± 3.03a,b	48.05± 3.50b	74 ± 5.40c

Results are mean±SD for 10 fishes. Values expressed: Na^+,K^+,Ca^+ µg/L. Values that have a different superscript letter (a, b, c) differ significantly with each other (P<0.05:Duncan's multiple range test).

Table 2. Levels of the activities of Mg^+, Na^+, K^+, and Ca^{2+} ATPase in the liver tissue (mg/g wet weight) of normal and experimental groups of fishes.

	Control (Group I)	Acetone (Group II)	Pesticide+acetone (0.3 µg/L) (Group III)	Pesticide+Acetone (1.1 µg/L) (Group IV)
Mg^+ ATPase	0.54 ±0.38a	0.5±0.36a	0.48 ± 0.34a	0.44± 0.31b
Na^+ ATPase	0.715 ± 0.51a	0.68± 0.48a	0.59± 0.42b	0.43 ±0.31c
Ca^{2+}ATPase	0.335± 0.24a	0.375 ± .27a	0.31± 0.22a	0.21 ± 0.15b

Results are mean ± SD for 10 fishes. Values expressed: Mg^+ATPase, Na^+ATPase, Ca^{2+}ATPase µg/L. Values that have a different superscript letter (a,b,c) differ significantly with each other (P<0.05:Duncan's multiple range test).

chains of the calcium channel and increases the probability of the calcium channel opening (Varadi et al., 1995). This may be the reason for enhanced activity of Ca^{2+} ATPase and increased concentration of Ca^{2+} observed in liver tissue of λ Cyhalothrin induced hepatotoxicity in fresh water fishes. Intracellular Ca^{2+} overload can set off a cascade of events that can lead to the formation of reactive oxygen species which suggest that reactive oxygen species formation and Ca^{2+} accumulation may be involved in the dysfunction of the liver (Jan et al., 2005). Since Ca^+ and Na^+ ions are competitive at a number of membrane sites, a high concentration of calcium ions in the cells of liver would compete with sodium-specific sites at the inner surface of the membrane, leading to a decrease in liver sodium content (Vicenzi, 1971).

Significantly (P<0.05) elevation observed in the level of Na^+ ions in group I (control) compared with group IV (1.1 µg/L) fishes (Table 1). A significant (P<0.05) elevation in the level of K^+ ions is found in group (IV) fishes (1.1 µg/L) compared with group I (control) fishes (Table 1). The transport of Na^+ and K^+ intra and extra pools and the maintainance of the transmembrane gradients are important to cell function and integrity. λ cyhalothrin-induced alteration in Na^+/K^+ co-transport mechanism might have played an important role in the Na^+/K^+ imbalance and intracellular calcium overload (Lang, 2007). λ cyhalothrin treatment has probably altered Ca^{2+} homeostasis by modulating the efflux of Ca^{2+} via the Na^+/Ca^{2+}exchanger (Ruhfus et al., 1996). In addition to this, the ability of λ cyhalothrin to change the integrity of membrane-bound ATPases might have contributed significantly to its role in maintaining the ionic equilibrium in fishes. This is in agreement with studies by Coelho-Sampaio et al. (1994). λ Cyhalothrin is also reported to de-normalize the content of potassium and calcium ion in piscian cells (Lang et al., 1998).

A significant (P<0.05) decline noticed in the activities of the membrane-bound ATPases (Na^+, K^+,- ATPase, Mg^{2+}-ATPase, and Ca^{2+}-ATPase) in the liver tissue of group IV (1.1 µg/L) fishes compared with group I (control) fishes (Table 2).This is in line with previous findings (Farvin et al., 2005). The Na^+/K^+ ATPase pump is responsible for the active transport of Na^+ and K^+ across the cell membrane *(*Ahmed and Thomas*,* 1971) reported that increased concentrations of free fatty acids (FFAs) in the hepatic cells resulted in the non-competitive inhibition of many enzyme systems such as Na^+/K^+ ATPase. Inhibition of the sodium pump may precipitate increased levels of intra cellular sodium (Jennings et al., 1986). The increased levels of FFAs may have resulted in non-competitive inhibition of Na^+/K^+ ATPase, there by leading to increased accumulation of Na^+ ions in λ cyhalothrin administered freshwater fishes. ATPases are integral membrane protein which requires thiol groups and phospholipids to maintain their structure and function according to Hazarika and Sarkar (2001), peroxidation of membrane phospholipids not only altered the lipid milieu and structural as well as functional integrity of cell membrane but also affected the activities of various membrane-bound enzymes including Mg^{2+} ATPase, Ca^{2+} ATPase, and Na^+, K^+ ATPase.

Activities of Na^+, K^+ ATPase, Mg^{2+}-ATPase and Ca^{2+}-ATPase in the liver were significantly lower in group IV (λ cyhalothrin induced toxic fishes) which is in line with previous reports (Sakaguchi and Yokota, 1995). The

Table 2. Levels of the activities of Mg^+, Na^+, K^+, and Ca^{2+} ATPase in the liver tissue (mg/g wet weight) of normal and experimental groups of fishes.

	Control (Group I)	Acetone (Group II)	Pesticide+acetone (0.3 µg/L) (Group III)	Pesticide+Acetone (1.1 µg/L) (Group IV)
Mg^+ ATPase	0.54 ±0.38a	0.5±0.36a	0.48 ± 0.34a	0.44± 0.31b
Na^+ ATPase	0.715 ± 0.51a	0.68± 0.48a	0.59± 0.42b	0.43 ±0.31c
Ca^{2+}ATPase	0.335± 0.24a	0.375 ± .27a	0.31± 0.22a	0.21 ± 0.15b

Results are mean ± SD for 10 fishes. Values expressed: Mg^+ATPase, Na^+ATPase, Ca^{2+}ATPase µg/L. Values that have a different superscript letter (a,b,c) differ significantly with each other (P<0.05:Duncan's multiple range test).

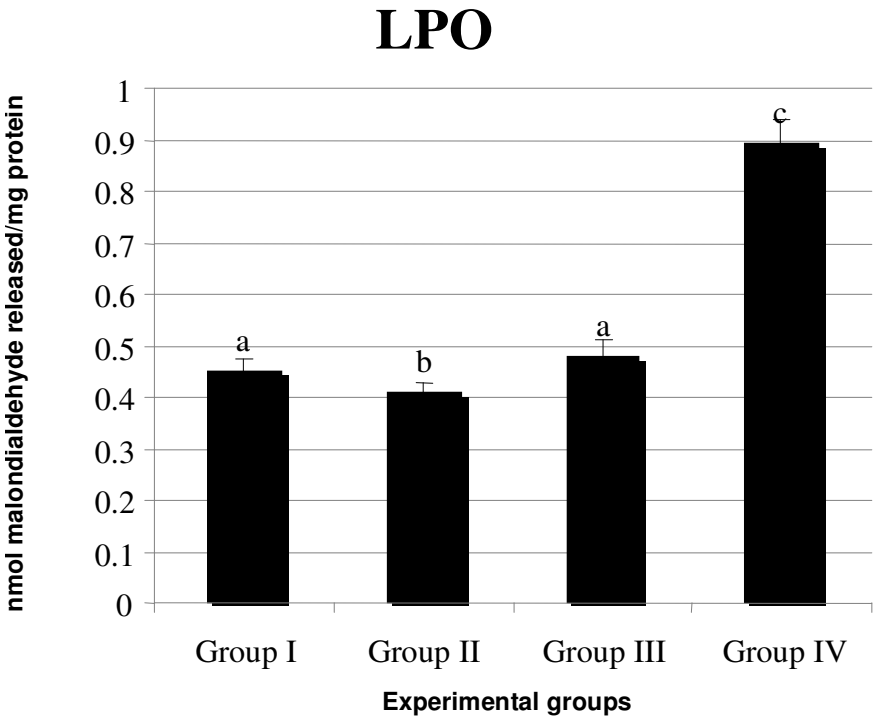

Figure 2. Group I (control), group II (acetone treated), group III (pesticide+ acetone (0.3 µg/L), group IV pesticide+ acetone (1.1 µg/L) for 15 days. Results are mean +SD for 10 fishes; one- way ANOVA; Duncan's multiple comparison test. Values that have a different superscript letter (a, b, c) differ significantly (p<0.05*)* with each other. Values expressed: LPO, nmol malondialdehyde released/mg protein.

intracellular concentration of calcium regulates the activity of the $Mg^{2+}$and Na^+, K^+-ATPases. Bironaite and Ollinger (1997) have reported that lipid peroxidation can influence the function of $Ca^{2+}$and Mg^{2+}-ATPases and the activity of membrane Ca^{2+-} translocase. The marked loss in the activities of membrane-bound ATPases may also be due to the loss of protein-SH, because of increased lipid peroxidative damage of cell membranes. The present study confirms that λ cyhalothrin induced alterations in the level of membrane-bound ATPase activity and minerals in fresh water teleost fish (*O. Mossambicus*). λ Cyhalothrin can be considered both as a potent oxidant and as a membrane de-stabilizer. It exerts cellular and subcellular membrane de-stabilization in the liver by modulating both non-enzymic and enzymic antioxidants (Kanbak et al., 2007).

Lipid peroxidation *in vivo* has been identified as one of the basic deteriorative reactions in cellular mechanisms of the λ cyhalothrin induced oxidative stress in fresh water fishes. In the present investigation, the administration of λ cyhalothrin induced a significant (P<0.05) increase in the level of lipid peroxidation in the liver tissue of group IV (1.1 µg/L) fishes as compared to group I (control) (Figure 2). This indicates the high vulnerability to peroxidative damage in λ cyhalothrin induced toxicity is probably due to a decline in the level of free radicals for scavengers. No significant alterations observed in groups II and III fishes as compared to group

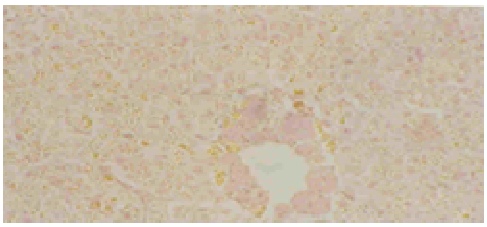

Plate 1. The architecture of normal liver tissue in group I (control fishes) (Haematoxylin and Eosin 100×).

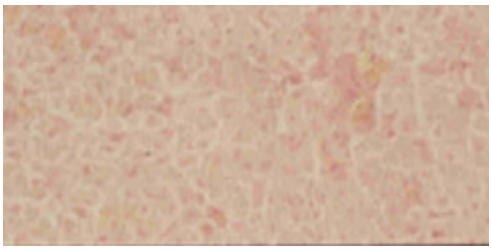

Plate 2. Architectural view in the liver tissue of λ cyhalothrin administered fishes group II (acetone treated) (Haematoxylin and Eosin 100×).

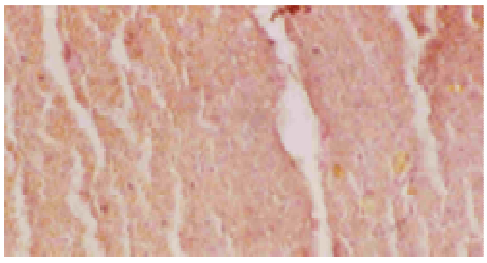

Plate 3. Photographic view of liver tissue of λ cyhalothrin administered fishes (group III) (pesticide + acetone 0.3 µg/L) Haematoxylin and Eosin 100×).

IV fishes. The parameters analyzed in these groups were comparable to that group I (normal control fishes).

Histopathological studies in liver tissue of Plate 1, group I (control) fishes illustrates less ceroid pigments and hepatocytes are well organized. Plate 2 architectural views in the liver tissue of acetone administered fishes, no much significant histopathological alterations in group II fishes were noticed as compared to group IV fishes. Plate 3 photographic view of liver tissue of λ cyhalothrin administered fishes group (III) (pesticide + acetone 0.3µg/L) shows vacoulation and swelling of the hepatocytes and a lot of ceroid pigments are also seen. Plate 4 histology of the liver tissue sections of group IV (pesticide+acetone1.1 µg/L λ cyhalothrin administered) fishes showed bilary epithelium proliferation and

Plate 4. Histology of the liver tissue sections of group IV (pesticide +acetone 1.1 µg/L λ cyhalothrin administered fishes (Haematoxylin and Eosin 100×).

vacoulation of hepatocytes. Vacoulation in the hepatocytes, bilary proliferation is prominent; moderate proliferation of bilary epithelium and appearance of ceroid pigments in the hepatocytes are also seen. This pathological aberration is probably related to a decline in oxygen supply with paramount rise in wall stress.

In conclusion, the result of the present study indicates that the λ cyhalothrin induced significant alteration in the mineral levels (sodium, potassium and calcium) and the activities of membrane-bound ATPases. It also elevates lipid peroxidation. The effect observed in this study is due to membrane destabilizing property or peroxidative action.

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