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Studies on the changes in lipid profile of λ Cyhalothrin- induced hepatotoxicity in fresh water fish *Tilapia* (*Oreochromis Mossambicus*)

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ABSTRACT

An attempt has been made to determine the deleterious effects of λ cyhalothrin-induced in fresh water tilapia (O.mossambicus) with respect to changes in the activities of lipids in the liver. Significant (p<0.05) elevation in the level of lipid peroxidation and in the level of cholesterol was observed in λ cyhalothrin-intoxicated fishes as compared to controls. Significant (p<0.05) decline was also observed in phospholipid and free fatty acids in λ cyhalothrin treated fishes as compared to controls. The result of the present study indicated that the overall hepatotoxic effect of λ cyhalothrin is probably related to lipid accumulation by its hyperlipidemic nature and/or the formation of free radicals by its oxidative character.

Key words: Cholesterol, free fatty acid, hepatotoxicity, λ cyhalothrin, lipid peroxidation, *O.mossambicus*, phospholipids.

INTRODUCTION

Pesticides are a broad term, covering a range of products that are used to control pests. Pesticides are classified into insecticides (insects), fungicides (fungi), bactericides (bacteria), herbicides (weeds), acaricides (mites or ticks), miticides (mites), and rodenticides (rodents) etc.

λ cyhalothrin belongs to the chemical family synthetic pyrethroid insecticide, with a chemical name (S)-α-cyano-3- = phenoxy benzyl(z) –, the IUPAC name (IR,3R) -3- (2-chloro-3,3,3-trifluoro propenyl)-2,2- = dimethyl cyclo propane carboxylate and (R)-α-cyano-3- phenoxy benzyl(Z)-1s,3s)-3-(2-=chloro-3,3,3,-trifluoropropenyl)-2,2- dimethyl cyclo propane carboxylate **(Figure 1)**.

Pyrethroids are a group of man made pesticides similar to the natural pesticide pyrethrum, which is produced by chrysanthemum flowers. The synthetic pyrethroids are stable and persist in the environment much longer than pyrethrin. Sunlight breaks down λ cyhalothrin in water and soil **[33, 34]**. Reported field half lives range from 4 to 12 weeks **[30, 31, 37]**. λ cyhalothrin has a low potential to contaminate ground water due to its low water solubility and high potential to bind to soil **[32]**. Pyrethroid insecticides are extremely toxic to most aquatic organisms. The use of organochlorine pesticide have 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.

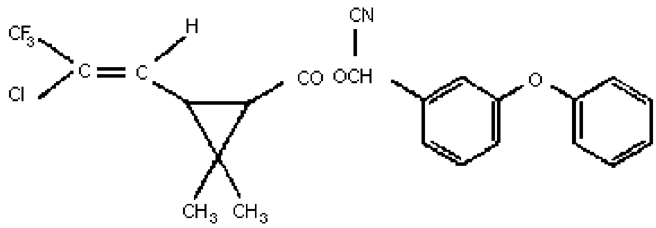

Fig :1 Chemical structure of λ Cyhalothrin

Fish are relatively sensitive to changes in their surrounding environment including an increase in pollution. *O.mossambicus* comes under the class: *actinopterygii (ray-finned fishes)*, order: *Perciformes* (perch-like), family: *Cichlidae* (Cichlids), species: *O.mossambicus*, common name: *Mozambique tilapia* (Afr.Bloukurper). The tilapias are characterized by a general tolerance to a wide range of environmental conditions including salinity **[20]**.

Changes in biochemical constituents of tissues and enzyme activity are important in order to determine the nature and extent of toxicant effects on organisms **[17, 9]**. The enzymic and nonenzymic biomarkers of oxidative stress can be sensitive indicators of aquatic pollution. Exposure to λ cyhalothrin on metabolic and morphologic aberrations in the liver tissue of the fishes not yet explored. In the present study, we have investigated the deleterious effects of λ cyhalothrin-induced hepatotoxocity in Fresh Water Tilapia (*O.mossambicus*) with respect to changes in the levels of cholesterol, phospholipids, free fatty acids 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 9-13cm and weight 2-7 gms 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 ± 2^0c, 12 h light/ dark cycle).

Experimental Protocol

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

Biochemical assays

Total cholesterol in the liver tissue was estimated as per the method **[23]**. Tissue lipid peroxidation level was determined as TBA-reactive substances by the method **[18]**. Phospholipids contents of liver is estimated by the method **[7]** as inorganic phosphorus liberated after digesting with perchloric acid as outlined in **[2]**. Free fatty acids in the liver tissue were estimated by the modified method **[11].**

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.

RESULT AND DISCUSSION

Cholesterol is required to build and maintain membranes; it modulates membrane fluidity over the range of physiological temperatures. Within the cell membrane, cholesterol also functions in intracellular transport, cell signaling and nerve conduction. Cholesterol is oxidized by the liver into a variety of bile acids. In the present investigation, a significant (p<0.05) elevation observed in the level of total cholesterol content in Group IV fishes compared to Group I control fishes. There are no significant changes in cholesterol content observed in Group III fishes and Group I control fishes. But a significant decline was observed in the level of cholesterol in Group II fishes (**Table 1)**. The reduced cholesterol level may be due to the inhibition of cholesterol biosynthesis in the liver or due to reduced absorption of dietary cholesterol as reported by **[13, 14]**. As per **[25]** reported that the cholesterol decrease may be due to utilization of fatty deposits instead of glucose for energy purpose. The present investigation is also in agreement with these findings.

Phospholipids act as building blocks of the biological cell membranes in virtually all organisms. They participate in the transduction of biological signals across the membrane. In the present investigation, there was a significant (p<0.05) elevation observed in the level of phospholipids content in Group II fishes when compared to Group I control fishes. There is a decrease in the level of phospholipids in Group III fishes compared to Group I control fishes. There is a significant (p<0.05) decline in the level of phospholipids content in Group IV fishes compared to Group II acetone treated fishes **Table 1**. Fall in phospholipids concentration is supported by the findings **[19]**, **[29]** in *Cyprinus carpio*, **[27]** in *Clarias batrachus* exposed to carbaryl, **[22]** in *Tilapia Mossambica*, **[5]** after nickel administration in *Channa punctatus*, **[21]** in *Cyprinus carpio*, **[26]** in *Cyprinus carpio*, **[4]** after petroleum oil treatment in *Heteropneustes fossilis*, **[6]** in *Labeo rohita*, **[24]** in *Heteropneustes fossilis* after endosulphan treatment and **[28]** in *Channa punctatus* respectively. Lipid peroxidation involves the direct reaction of oxygen and lipid to form free radical intermediate and to produce semistable peroxide. Pathological free radical mechanism leading to lipid peroxidation and degradation of phospholipids with loss of membrane integrity are currently considered to be an important factor [1].

Free Fatty acids are important sources of fuel because their metabolism yields large quantities of ATP. In the present study a significant (p<0.05) increase observed in the levels of fatty acid content in Group IV fishes compared to Group I control fishes and Group III fishes. There is a significant (p<0.05) decline in the levels of fatty acid content observed in Group II compared with Group IV fishes (**Table 1)**. Fatty acids are amphipatic molecules and are known to destabilize biomembranes **[10, 15, 12]**. Biological membranes are sensitive to lipid peroxidation induced by reactive oxygen species. Due to inhibition of the lipid oxidation fatty acids and their metabolites will accumulate rapidly **[16]**. The liver damage is due to the over production of free radicals during lipid metabolism which induce an oxidative stress state. Lipid peroxidation causes decrease in membrane fluidity and barrier functions of membranes [3].

Table-1 Level of lipid components in the liver tissue of control and experimental groups of fishes

Parameters	Control Group-I	Acetone treated Group-II	Pesticide+Acetone 0.3µg/l Group-III	Pesticide+Acetone 1.1µg/l Group-IV
CHOLESTEROL	3.10 ± 0.22^{a}	2.88 ± 0.20^{b}	3.45 ± 0.24^{a}	6.15 ± 0.45^{c}
PHOSPHOLIPIDS	12.1 ± 0.88^{a}	13.25 ± 0.98^{b}	10.3 ± 0.78^{a}	7.45 ± 0.55^{c}
FREE FATTY ACIDS	0.09 ± 0.06^{a}	0.06 ± 0.42^{bc}	0.085 ± 0.56^{c}	0.16 ± 0.01^{ab}

Results are mean + SD for 10 fishes. Values expressed: Cholesterol, Phospholipids and Fatty acids mg/g wet tissue. Values that have a different superscript letter (a,b,c) differ significantly with each other (p<0.05:Duncan's multiple range test).

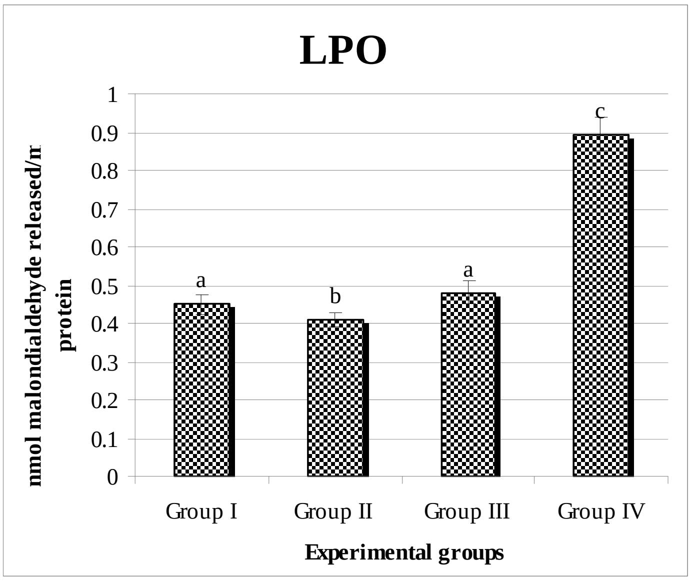

Fig :2 Group I control, Group II acetone treated, Group III pesticide+ acetone (0.3µg/l), Group IV pesticide+ acetone (1.1 µg/ l) for 15 days Results are mean +SD for 10 fishes; one- way ANOVA; Duncan's multiple comparison test. Values that have a different superscript letter (a, b, c) differ significantly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

Group I, Control, Group II, acetone treated, Group III, λ cyhalothrin dissolved in acetone (0.3µg/l) for 15 days: Group IV, λ cyhalothrin dissolved in acetone (1.1µg/l) for 15 days

Lipid Peroxidation refers to the oxidative degradation of lipids. It is a process by which free radicals steal electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. The reaction consists of three main steps: initiation, propagation and termination. Fatty acid radical is being produced in the initiation step. Initiators in living cells are reactive oxygen species (ROS) like OH and HO2, they combine with hydrogen atom to make water and fatty acid radical. The fatty acid radical reacts with molecular oxygen thereby producing a peroxy fatty acid radical. This reacts with another free fatty acid producing a different fatty acid radical and lipid peroxide. The radical reaction stops when two radicals react and produce a non radical species. 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 1.1µg/l fishes as compared to Group I control. 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. Antioxidants are necessary for preventing the information of free radicals and they inhibit some of the deleterious actions of reactive oxygen species that damage lipids, DNA and proteins **[8]**. There were no significant alterations observed in the level of lipid peroxidaion in Group II and Group III fishes as compared to Group I fishes (**Figure 2)**. Lipid peroxidation is a complex sequence of reactions that causes the disruption of cell membrane functions. λ cyhalothrin administered fishes might be less resistant and more susceptible to lipid peroxidation.

In conclusion, the present study indicate that the hepatotoxic effect induced by λ cyhalothrin may probably be due to lipid accumulation by its hyperlipidemic property/ or to activate the free radicals by its oxidative character.

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