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Study on the changes in the levels of protein metabolism in λ cyhalothrin-induced hepatotoxicity in fresh water *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 level of protein in the liver. Significant ($p<0.05$) elevation in the level of lipid peroxidation was observed in Group IV [pesticide+ acetone 1.1μg/l] fishes as compared to controls. Significant ($p<0.05$) decline was also observed in protein content in Group IV [pesticide+ acetone 1.1μg/l] fishes as compared to controls. The result of the present study indicated that λ cyhalothrin elevates lipid peroxidation [LPO], consequently oxidative stress also induces changes in free radical production. As a result when the free radical production overwhelms the endogenous antioxidant levels, they cause considerable cell damage and death. Depletion of protein fraction in liver tissues may have been due to their degradation and possible utilization for metabolic purposes.

Key words: Hepatotoxicity, λ cyhalothrin, lipid peroxidation, *O. mossambicus*, oxidative stress, protein.

INTRODUCTION

Synthetic chemical pesticides are used because of their vigorous and outstanding results. But their disproportionate use causes significant loss to ecosystem-terrestrial as well as aquatic and subsequently the flora and fauna of surrounding. λ cyhalothrin, an insecticide, belongs to the pyrethroid chemical class of pesticides. Pyrethroids are a group of man made pesticides similar to the natural pesticide pyrethrum, which is produced by chrysanthemum flowers. λ cyhalothrin, a synthetic pyrethroid insecticides targets a wide range of insects including aphids, Colorado beetles, and butterfly larvae. It is also used to control insects identified as potential disease vectors, such as cockroaches, mosquitoes, ticks, and flies. In commercial applications, λ cyhalothrin is used on food crops (vegetables and fruits) non-food crops (cotton) in green houses, in and around hospitals, for cattle (in ear tags), and in termite treatments. Residential use can be both indoors and outdoors on homes, ornamental plants, and lawns.

λ cyhalothrin is highly toxic to many fish and aquatic invertebrate species [11, 30]. Lobster, shrimp, mayfly, nymphs and zooplanktons are the most susceptible non-target aquatic organisms [18]. LC_{50} for blue gill sunfish is 0.21µ g /l λ cyhalothrin, and for rainbow trout is 0.24µg/l λcyhalothrin, where as the LC_{50} value for λ cyhalothrin exposure on *O.mossambicus* is found to be 1.3 µg/l. λ cyhalothrin is highly toxic to bees [30]. λ cyhalothrin`s toxicity to birds ranges from slightly toxic to practically nontoxic.

Fish tend to lack the enzymatic machinery for the metabolism of this pyrethroid which is the obvious reason for the deleterious effect of this pesticide on fish [5].Tilapia is highly resistant to diseases, tolerant to poor water quality [3]. *O. mossambicus* is economically important for the following reasons: fisheries [highly commercial], aquaculture [commercial], game fishing as recreational aquarium fish [commercial] and are also used extensively in biological, physiological, and behavioral research [28].

λ cyhalothrin induced metabolic and morphologic aberrations in the liver tissue of the fishes is not yet explored. Oxygen free radicals are reportedly involved in toxicity of numerous chemicals and also in pathogenesis of many diseases, [24].

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

The fishes were grouped into four of 10 fishes each after being acclimatized, 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. Tanks were aerated and covered with nylon nets. The toxicant solution was changed every 24h and the experiment was continued for a period of 15 days. At the end of the experiment, the live fishes were sacrificed and the liver tissue excised was used for the determination of protein [15] and lipid peroxidation [21].

Biochemical assays

Protein in the liver tissue was estimated as described by Lowry *et al* [15]. Tissue lipid peroxidation level was determined as TBA-reactive substances by the method of Ohkawa [21].

Statistical analysis

All data were analyzed using ANOVA with the aid of SPSS 10.0 for windows. Data obtained were expressed as mean $\pm$ SD. Multiple comparisons of the means were separated using the Duncan Multiple Range Test at 5% probability.

RESULTS AND DISCUSSION

Proteins are involved in the architecture and physiology of the cell and also in cell metabolism [16]. Proteins [also known as polypeptides] are organic compounds made of amino acids arranged in a linear chain and folded into a globular form. Protein metabolism is considered the most sensitive physiological responding to environmental stress.

Protein synthesis and protein degradation are highly regulated cellular processes essential for cell viability. In the present investigation, there was a significant ($p<0.05$) increase was observed in the level of protein content in Group II fishes as compared to Group I fishes. But a significant decline was noticed in the level of protein contents in the liver tissue of Group IV. There was no significant changes in protein content observed in Group I control fishes and Group III fishes **Fig 1**.

The significant depletion of protein content observed in Group IV fishes may be due to their degradation and also to the possible utilization of these compounds for metabolic purposes. These results are in agreement with the earlier findings which indicated that the decreased protein content might also be attributed to the destruction/necrosis of cells and consequent impairment in protein synthetic machinery. According to Nelson *et al* [20], the physiological activity of animal was indicated by the metabolic status of proteins. Protein being involved in the architecture and physiology of the cell, they seem to occupy a key role in cell metabolism [19]. Catabolism of proteins and amino acids make a major contribution to the total energy production in fishes. Jrueger *et al* [9] reported that the fish can get its energy through the catabolism of proteins. Thus the depletion of protein fraction in liver tissues may have been due to their degradation and possible utilization for metabolic purposes.

The depletion of protein content observed in this investigation can be correlated to this fact. These results are in agreement with the earlier report of David *et al* [4] who demonstrated a similar situation in *Ciprinus Carpio* exposed to cypermethrin. Depletion of tissue protein in fishes exposed to toxicants has been reported by several workers by [24] suggested that the pesticide stress influences the conversion of tissue protein into soluble fraction reaching in the blood for utilization. The reduction in proteins may be due to increased energy demand during stress or it could be due to altered enzymatic activities [14]. In long term exposure to λ cyhalothrin much of the energy must have been used up to compensate the stress, hence the depletion in the protein content is observed.

An alteration of protein metabolism was observed in fish exposed to various types of environmental stresses like metals and pesticides, [1, 26]. Stress proteins are considered to be general indicators of sub lethal cellular protein damage. The quality of protein is dependent on the rate of protein synthesis, or on rate of its degradation. The quality of the protein may also be affected due to impaired incorporation of amino acids into polypeptide chain [22, 25] suggested that the fish exposed to pesticides may compensate any possible protein loss by increasing its protein synthesis. Gill *et al* [7] concluded that compensatory production of enzymes lost as result of tissue necrosis or to meet increased demand to detoxify the pesticides might have necessitated enhanced synthesis of enzyme proteins. Increase in free amino acid levels were the result of break down of protein for energy and impaired incorporation of amino acids in protein synthesis.

The toxicants may affect the hormonal balance which could directly or indirectly affect the tissue protein levels [27, 17, 12].

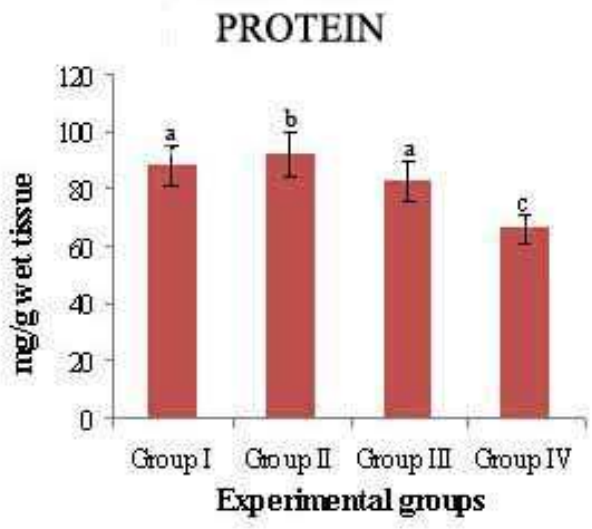

Fig. 1 Level of protein content in liver tissue of control and experimental groups of fishes Results are mean ± SD for 10 fishes; one-way ANOVA (p<0.05: Duncan's multiple range test). Values expressed: Protein mg/g. Values that have a different superscript letter (a,b,c) differ significantly with each other.

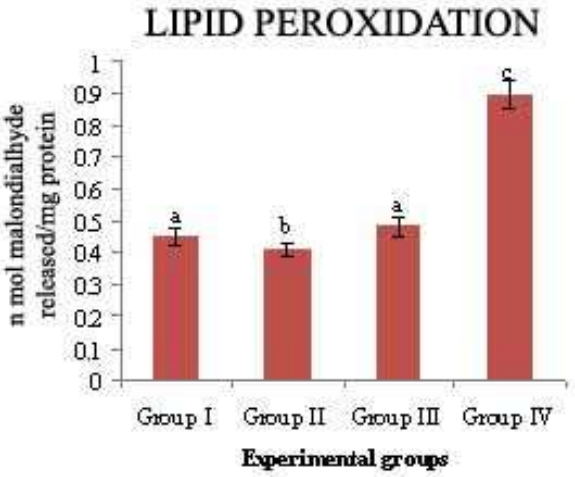

Fig.2 Level of LPO content in liver tissue of control and experimental groups of fishes Results are mean ± SD for 10 fishes; one-way ANOVA (p<0.05: Duncan's multiple range test). Values expressed: LPO µmol malondialdehyde released/ mg protien. Values that have a different superscript letter (a,b,c) differ significantly with each other.

Understanding of the protein components of cell becomes necessary in the light of the radical changes that take place in protein profiles during pesticide intoxication. Both the protein degradation and synthesis are sensitive over a wide range of conditions and slow changes to a variety of physical and chemical modulators. The degradation is due to oxidative stress [10], which is a characteristic of organophosphate compounds, besides their inhibitory effect on acetyl cholinesterase. Oxidative stress also induces changes in free radical production. When the free radical production overwhelms the endogenous antioxidant levels, they cause considerable cell damage and death. All the major biomolecules like lipids, proteins and nucleic acids may be attacked by free radicals [2]. The physiological and biochemical alterations observed in an animal under any physiological stress can be correlated with the structural and functional

changes of cellular proteins. Proteins occupy a unique position in the metabolism of cell because of the proteinacous nature of all the enzymes which mediate at various metabolic pathways [13, 8]. A Possible role of λ cyhalothrin toxicity on protein metabolism is tried to illustrate in **Fig. 3**

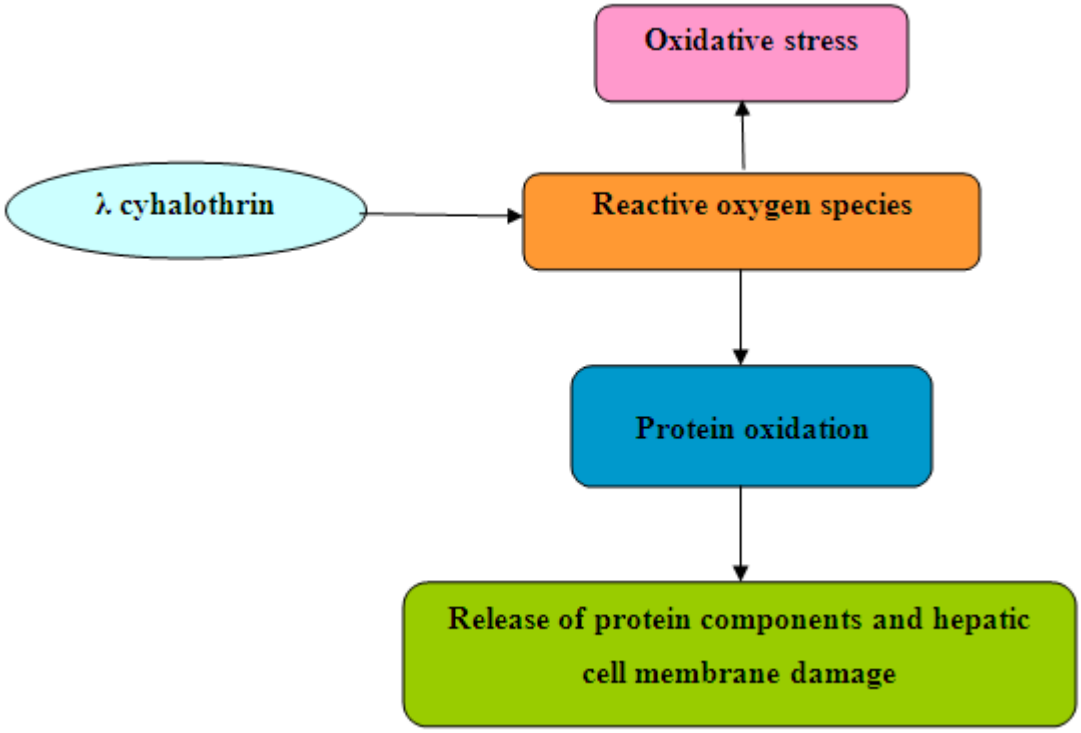

Fig.3 Possible role of λ Cyhalothrin toxicity on protein metabolism

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 **Fig. 2**. Oxygen radicals catalyse the oxidative modification of lipids [6]. The presence of double bond adjacent to a methylene group makes the methylene C-H bonds of polyunsaturated fatty acid [PUFA] weaker and thereore the hydrogen becomes more prone to abstraction. While lipid peroxidation is not initiated by O^{-}_{2} and H_2O_2, $^{\cdot}H$, alkoxy radicals ($RO^{\cdot}$), and peroxy radicals ($ROO^{\cdot}$) result in initiating the lipid peroxidation [29]. This can lead to a self perpetuating process since peroxy radicals are both reaction initiators as well as the product of lipid peroxidation. Lipid peroxy radicals react with other lipids, proteins and nucleic acids; propagating there by the transfer of electrons and bringing about the oxidation of substrates.

In conclusion, the present study indicates that λ cyhalothrin elevates lipid peroxidation. As a result when the free radical production overwhelms the endogenous antioxidant levels, they cause considerable cell damage and death. Depletion of protein fraction in liver tissues may have been due to their degradation and possible utilization for metabolic purposes.

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