5. DISCUSSION

Pyrethroids are synthetic insecticides structurally based on the pyrethrins, which are botanical insecticides (Soderlund et al., 2002). Pyrethroid insecticides have been used for more than 40 years in view of their wide availability, and consequently accounting for 25% of the world insecticide market (Kakko et al., 2003 and Shafer et al., 2005). As such, their use has risen dramatically over the past 10 years in India (Ramesh and Vijayalakshmi 2001). Available literature suggests that indoor pyrethroid exposure is of considerable magnitude in India and other countries including the United States (Bateman 2000; Pankaj and Prahalad 2004; Narahashi 2000) as a result of the wide-spread use of pyrethroid based repellents to control a variety of pests such as mosquitoes and cockroaches (Narahashi 2000; WHO 2000) due to their high insecticidal and low mammalian acute toxic effects (Kakko et al., 2003). Though severe toxicity of pyrethroids has been uncommon in developed countries, it appears to be common in developing countries because of their extensive and intensive use for agricultural and domestic purposes (Kako et al., 2003; Shafer et al., 2005; Bateman 2000).

Synthetic pyrethroid insecticides are extensively used to replace organochlorine, organophosphates and carbamates to control various types of pests and increase agricultural products. Unfortunately, these chemicals are also found to be potentially
toxic to aquatic species (Reddy and Bashamohideen, 1998). According to Beat et al., 1997, all pyrethroid insecticides are neurotoxic and act on the axons in the peripheral and central nervous system. Pyrethroids are lipophilic in nature and therefore have high rate of gill absorption which contributes to the sensitivity of fish to their exposure (Rukiya et al., 2003).

Cells are protected against oxidation by the action of certain enzymes, vitamins and other substances, known collectively as antioxidants (Ishii and Fernandez-Checha 1997). As a consequence of the constant oxidative challenge, cells have evolved antioxidant systems to counter the proxidant fluxes. Notable among these antioxidant mechanisms are enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase. These enzymes react directly with the oxidizing radicals to yield non-radical product. When the balance between the ROS (reactive oxygen species) production and antioxidant defense is lost, “oxidative stress” results, which through a series of events deregulates the cellular functions leading to various pathological conditions (Fernandez-Checha et al., 1997).

Liver is the centre for metabolism and detoxification in piscine body (Athikesavan et al., 2006). Liver damage is the first indication of chemical induced toxicity as it encounters firstly by any oxidative stress. Liver plays an important role in metabolism by maintaining the energy level and exerting structural stability of tissues
(Guyton and Hall 2002). It is also a site of biotransformation by which a toxic compound has been transformed in less harmful form to reduce toxicity (Hodgson 2004). However, this will damage the liver cells and produce hepatotoxicity.

Pesticides and numerous environmental stressors may induce oxidative stress, leading to generation of free radicals and alteration in antioxidants, or oxygen free radical, the scavenging enzyme system, and lipid peroxidation (Akhgari et al., 2002). Generation of reactive oxygen species and the resulting damage may be mediated directly by a toxic compound and its metabolites, or by alteration of metabolic links, which indirectly increase the process of free radical generation (Liczmanski 1988; Bartosz 1995; Southern and Powis 1998). Also several studies have indicated that pyrethroids induce oxidative stress and lipid peroxidation (Gupta 1999; Gabbiarelli et al., 2002).

The ability of an organism to adapt to environmental alteration by industrial contamination depends mainly on the effecting mechanisms of detoxification of various endo and exogenous compounds, (Jakonovic 2001). The mechanism by which pesticides cause damage varied according to the structure of the pesticides.

5.1 Protein content in the liver tissue of Oreochromis mossambicus

Proteins are involved in the architecture and physiology of the cell and also in cell metabolism (Mommsen and Walsh 1992). 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 behavior responding to environmental stress.

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 (findings by Rekha Parthasarathy and John Joseph 2011). 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 (David et al. 2004). According to Nelson et al (2005), the physiological activity of fish 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 (Murray et al., 2007). Catabolism of proteins and amino acids make a major contribution to the total energy production in fishes. Jrueger et al (1968) reported that the fish can get its energy through the catabolism of proteins.

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., (2004) 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 (Ramalingam and Ramalingam, 1982) suggested that the pesticide stress influences the conversion of tissue protein into soluble fraction reaching 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 (Lett et al., 1976). 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 been observed.

An alteration of protein metabolism was observed in fish exposed to various types of environmental stresses like metals and pesticides, (Alexsandro et al., (2009); Sheweta and Gopal 2009). 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 the rate of its degradation. The quality of the protein may also be affected due to impaired incorporation of amino acids into polypeptide chain (Ram et al 2003). Salib et al (1984) suggested that the fish exposed to pesticides may compensate any possible protein loss by increasing its protein synthesis. Gill et al (1990) 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 impaire incorporation of amino acids in protein synthesis. The toxicants may affect the hormonal balance which could directly or indirectly affect the
tissue protein levels (Morthy and Priyamvada 1982; Khilare and Wagh 1988; Singh et al., 1996).

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 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 (Hai et al., 1995), 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 bio molecules like lipids, proteins and nucleic acids may be attacked by free radicals (Cheeseman and Slater 1992). 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 proteinaceous nature of all the enzymes which mediate at various metabolic pathways (Lehninger, 2008; Harper, 2006). The alterations noted in the experimentally induced fishes might be due to the leakage of enzymes and protein bound components from the damaged liver tissue into the systemic circulation. A possible role of λ cyhalothrin toxicity on protein metabolism is illustrated in Fig 5.1.1
5.2 Influence of \( \lambda \) cyhalothrin on taurine in the liver tissue of *Oreochromis mossambicus*

The \( \alpha \)-amino acids together with \( \beta \)-amino acids taurine constitute a very significant proportion (67%) of the free amino acid pool in the liver tissue of fishes. Unlike other amino acids taurine is present at substantially higher concentration in the liver tissue of fishes.
5.2.1 Taurine

Taurine, or 2-aminoethanesulfonic acid, is an organic acid. It is a major constituent of bile and can be found in the lower intestine and in small amounts in the tissues of many animals including fishes. Taurine is a derivative of the sulphur-containing (sulfhydryl) amino acid cysteine. Taurine is one of the few known naturally occurring sulfonic acids. Taurine is a conditionally essential amino acid and is either derived from food/feed biosynthesised in the liver. Taurine is involved in various important biological and physiological functions, which include cell membrane stabilization (Heller-Stilb et al., 2002), antioxidation (Atmaca 2004), detoxication (Birdsall, 1998), osmoregulation (Timbell et al., 1995), neuromodulation, brain (Renteria et al., 2004) and retinal development (Wright et al., 1986). In fish, taurine is one of the main osmoregulators (O’Flaherty et al., 1997). Taurine has been demonstrated to function as a direct antioxidant that scavenges or quenches oxygen free radicals, thus inhibiting lipid peroxidation, and as an indirect antioxidant that prevents the increase in membrane permeability resulting from oxidant injury in many tissues including liver (Chen, 1993). On the other hand, taurine can also function as regulator of intracellular calcium homeostasis (Huxtable, 1992).

As an indirect antioxidant, taurine has been proposed as a membrane stabilizer that can maintain membrane organization, prevent ion leakage and water influx, and
subsequently, avoid cell swelling (Milei et al., 1992; Chen, 1993). In the present study, the stabilizing impact of taurine on cellular membrane has been proposed to be connected with the interaction between taurine and polyunsaturated fatty acids in the membrane, which brings about the increasing similarity of taurine for its carrier transport and the interaction between taurine and the areas associated with anion transport and water influx. The role of taurine in modulating calcium homeostasis could be of specific importance for pathological diseases that are characterized by excessive calcium overloads. In Group IV fishes λ cyhalothrin might have inhibited the taurine mediated biological processes such as cell membrane stabilization, antioxidation and osmoregulation in the hepatic membrane. This might be a possible reason for the λ cyhalothrin induced toxicity.

5.3 Lipid profile in the liver tissue of Oreochromis mossambicus

5.3.1 Cholesterol

Lipids constitute very rich energy reserve, its decrease indicates the changes in energy demand of fish during exposure to λ cyhalothrin. Lipid oxidation supplies the major part of energy requirement during slow swimming of fish (Bilinski and Beis 1975).

Cholesterol is an extremely important biological molecule that has roles in membrane structure as well as being a precursor for the synthesis of the steroid
hormones and bile acids. It is well known that fish under stress secrete high amounts of catecholamine which deplete glycogen reserves (Pickering 1981). Cell membranes are mainly constituted by specific lipids and proteins. The cholesterol level was found to be decreased in the exposure periods. Such a trend has also been reported by Palanichamy et al. (1986) in *O. mossambicus* on exposure to 3 different periods. The increased cholesterol level in liver tissue of Group IV fishes might be due to the increased mobilization of cholesterol from adipose tissue in to the liver. Jayantha Rao et al. (1984) and Kanagaraj et al. (1993). Shakoori et al. (1996) reported that the cholesterol decrease may be due to utilization of fatty deposits instead of glucose for energy purpose. Cholesterol accumulation may also be due to decreased utilization of fatty deposits for energy purpose (findings by Rekha Parthasarathy and John Joseph 2011). The synthesis and utilization of cholesterol must be strictly regulated in order to prevent over accumulation and abnormal deposition within the body. Cholesterol synthesis occurs in the cytoplasm and microsomes (ER) from the two carbon acetate group of acetyl-CoA. Increase in cholesterol content may indicate slow metabolism that resulted in total lipid contents.

5.3.2 Phospholipids

Phospholipid are a class of lipids and are a major component of all cell membranes as they can form lipid bilayers. Lipids are an important source of nutrition
in fish providing a significant amount of energy and structural components for reproductive growth (Sargent, 1995).

Lipid peroxidation involves the direct reaction of oxygen and lipid to form free radical intermediate and to produce semi stable 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. Fall in phospholipids concentration is supported by the findings of Naqvi et al (1988), Sivaramakrishna and Radhakrishna (1988) in *Cyprinus carpio*, Sharma (1999) in *Clarias batrachus* exposed to carbaryl, Rani et al (2001) in *Tilapia mossambica*, Desai (2002) after nickel administration in *Channa punctatus*, Radha et al (2005) in *Cyprinus carpio*, Shanthi et al (2005) in *Cyprinus carpio*, Borah (2005) after petroleum oil treatment in *Heteropneustes fossilis*, Dutta et al (2005) in *Labeo rohita*, Singh and Singh (2007) in *Heteropneustes fossilis* after endosulphan treatment and Shukla et al (2007) in *Channa punctatus* respectively. Most phospholipids contain a diglyceride, a phosphate group and a simple organic molecule such as choline. It appears that \( \lambda \) cyhalothrin impair the stability of the cell membrane by damaging its structural lipid by peroxidation decomposition, which may lead to subsequent cell necrosis and functional derangement. Peroxidation degradation of phospholipids would lead to alteration in the configuration and function of membranes and may thus alter their permeability.
characteristics (findings by Rekha Parthasarathy and John Joseph 2011). Decrease in membrane cholesterol, total phospholipids concentrations and membrane lipid peroxidation with no change in the membrane protein on exposure to λ cyhalothrin in *O. mossambicus* suggested no change in membrane fluidity. However it lead to a significant change in lipid packing in membrane, facilitating the easy entry of the pyrethroid into the bilayer which may lead to increased interaction among membrane constituents and also with the pyrethroid. The fluidity of the membrane has been shown to depend mainly on cholesterol content and the orientation of lipid molecule and their composition of fatty acyl chains in the membrane. Lipophilic nature of the pyrethroid facilitates its miscibility with the hydrophobic moiety and probably by forming pyrethroid-phospholipids mixture patches (aggregates/domains) and thereby substituting the depleted lipid content and restoring the basic structure and physical state of the bilayer without affecting fluidity of the bio membranes (Moya quile *et al.*, 1995).

### 5.3.3 Free fatty acid

Fatty acids are acids produced when fats are broken down. The principle components of most lipids are fatty acids (Castell, 1981). These acids are not highly soluble in water and they can be used for energy by most types of cells. They may be monounsaturated, polyunsaturated or saturated. They are organic, or in other words
they contain both carbon and hydrogen molecules. Fatty acids help to move oxygen through the blood stream to all parts of the body. Fatty acids are amphipatic molecules and are known to destabilize bio membranes (Hoekstra and Golovina 2002; Kartz and Messineo 1981; Hutter and Soboll 1992). They aid cell membrane development, strength and function, and they are necessary for strong organs and tissues. Due to inhibition of the lipid oxidation fatty acids and their metabolites will accumulate rapidly (Moore 1985). Biological membranes are sensitive to lipid peroxidation induced by reactive oxygen species. The Liver damage is due to the over production of free radicals during lipid metabolism which induce an oxidative stress state. The oxidation of unsaturated fatty acids in biological membranes may cause impairment of membrane function, decrease in membrane fluidity, inactivation of membrane receptors and enzymes, increase of non-specific permeability to ions and disruption of hepatic membrane structure (Rekha Parthasarathy and John Joseph 2011). Free radical scavenging enzymes such as catalase, superoxide dismutase, glutathione peroxidase and glutathione -s-transferase are the first line of cellular defence against oxidative injury, decomposing O2 and H2O2 before interacting to form the more reactive hydroxyl radical (OH'). The equilibrium between these enzymes is an important process for the effective removal of oxygen stress in intracellular organelles.
5.4 Lipid peroxidation as an indicator of free radical damage in the liver tissue of *Oreochromis mossambicus*

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. Free radical induced lipid peroxidation results in the deterioration of biological membranes (Sen *et al.*, 2006). In the present investigation the administration of λ cyhalothrin induced a significant increase in the level of lipid peroxidation in the liver tissue of Group IV fishes as compared to Group I control. This indicates that 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 formation of free radicals and they inhibit some of the deleterious actions of reactive oxygen species that damage lipids, DNA and proteins (Haidara *et al.*, 2006). There were no significant alterations observed in the level of lipid peroxidation in Group II and Group III fishes as compared to Group I control fishes. Reports by (Anandan *et al.*, 2004) showed that lipid peroxidation is a complex sequence of reactions that leads to the disruption of membrane functions. Our results also confirmed the same pattern and showed that λ cyhalothrin exposed fishes might be less resistant and more susceptible to lipid peroxidation.
Three different mechanisms are able to induce lipid peroxidation: autoxidation (by free radical reaction), photo-oxidation and enzyme action. Autoxidation is a radical-chain process involving 3 sequences, initiation, propagation and termination. The general process of lipid peroxidation consists of three stages: initiation, propagation and termination. Initiation occurs when oxygen is partly reduced by Fe$^{2+}$ to species able to abstract a hydrogen atom from a methylene carbon. Resulting alkyl radical reacts rapidly with oxygen to form a peroxy radical (LOO$^\cdot$), which itself can liberate LOOH via hydrogen abstraction from a neighbouring allyl bond. In this reaction new alkyl radicals are produced which propagate lipid peroxidation.

$$n\text{Fe}^{2+} + \text{O}_2 \rightarrow n\text{Fe}^{3+} + \text{reduced O}_2$$  \hspace{1cm} (I)

$$I+\text{LH} \rightarrow \text{IH}+\text{L}$$  \hspace{1cm} \text{initiation}

$$\text{L}+\text{O}_2,\text{LOO}^\cdot$$

$$\text{LOO}^\cdot+\text{LH} \rightarrow \text{LOOH}+\text{L}$$  \hspace{1cm} \text{Propagation}

Fe$^{2+}$ can substantially enhance lipid peroxidation by decomposing LOOH to highly reactive lipid alkoxy radicals (LO) that behave as organic initiators and branch lipid peroxidation.

$$\text{Fe}^{2+}+\text{LOOH} \rightarrow \text{Fe}^{3+}+\text{OH}^\cdot+\text{LO}$$
LO+LH→LOH+L

chain branching

Excess Fe$^{3+}$ can also complete, as electron donors, for LOO and LO inhibiting both the propagation and chain branching reactions and causing the Fe$^{2+}$ dependent termination of lipid peroxidation.

Fe$^{2+}$+LOO/LO→Fe$^{3+}$+LOOH/LOH

termination

The results of the present study demonstrated that λ cyhalothrin might have stimulated lipid peroxidation by influencing a variety of these reactions. λ cyhalothrin might have enhanced the initiation process not only by producing OH$^-$ but also by activating the Fe$^{2+}$ autoxidation. This action of λ cyhalothrin may alter other molecules of biological relevance in cellular and sub cellular membranes. λ cyhalothrin might have activated the Fenton-like reaction that causes the formation of the alkoxy radicals initiator of lipid peroxidation. It elevates the amount of Fe$^{2+}$ oxidized probably by acting with a site specific mechanism similar to that described for other OH$^-$ producers stimulating the Fenton reaction. The alteration of redox recycling of iron, affects the Fe$^{2+}$/Fe$^{3+}$ ratio in the reaction mixture. Both these phenomena may account for the activations exerted by λ cyhalothrin on the peroxidation of cell membranes. As there is some evidence that λ cyhalothrin alters the Ca$^{2+}$ binding sites on membrane acidic phospholipids, in particular of the phosphatidylserine and phosphatidylinositol classes.
λ cyhalothrin may activate peroxidation by enhancing cellular Ca\textsuperscript{2+} as it does for other Ca\textsuperscript{2+} dependent processes. Fig 5.4.1 illustrates hepatic antioxidant defence system in λ cyhalothrin-induced oxidative stress in fresh water fish Tilapia (O. mossambicus).

Fig 5.4.1 Hepatic antioxidant defense system in λ cyhalothrin-induced oxidative stress in fresh water tilapia (Oreochromis mossambicus)

5.5 Influence of λ cyhalothrin on antioxidant activities in the liver tissue of O. mossambicus

Oxidative stress arising from over production of ROS and the break down of antioxidant defenses is documented to induce chromosomal breakage and formation of
bone marrow micronuclei (Simic 1994). It has been suggested that clastogenic factors are released by cells exposed to oxidative stress (Emerit et al., 1995). Pyrethroids can affect cells by generating oxidant stress. This process is accompanied by a response of the antioxidant system of the cells, manifested by a change in the activity of the antioxidant enzymes, such as superoxide dismutase, and catalase, and by change in the concentration of antioxidants (Giray et al., 2001, Kale et al., 1999).

5.5.1 Glutathione

Glutathione is one of the abundant tripeptide nonenzymatic biological antioxidants present in the liver (Anderson, 1998). GSH is a key component of the cellular defense against injury and lipid peroxidation damage and a co-factor for the activity of GST’s. These have a crucial role in the termination of free radical cascades and the lipid peroxidation chain reaction (Mark et al., 1997). It acts as a substrate for H$_2$O$_2$ removing enzyme glutathione peroxidase and for dehydroascorbate reductase (Ahmed and Khater 2001). It also plays a critical role in cellular function, which includes the maintenance of membrane protein, the removal of free oxygen radicals such as peroxyl radical, superoxide radical, alkoxy radical, translocation of amino acids across cell membranes, the detoxification of foreign compounds and biotransformation of drugs (Comporti et al., 1991);(Muriel et al., 1992). In the present study a significant decline in the level of GSH in Group IV fishes was observed compared to Group I.
normal control fishes. The tissue antioxidant status might be operating at diminished level in \( \lambda \) cyhalothrin induced fishes. Reduction noticed in the level of GSH in \( \lambda \) cyhalothrin induced fishes was either due to increased degradation or decreased synthesis of glutathione. GSH protects the hepatocellular membranes from the oxidative damaging action of lipid peroxides.

5.5.2 Glutathione dependent antioxidant enzymes

GSH-GPx catalyzes the peroxides and reduces the glutathione to form oxidised glutathione and water (Rayman 2000). Glutathione-S- transferase is a major family of detoxification enzymes. GST’s plays a vital role in protecting tissues against oxidative damage and oxidative stress. The GST catalyzes the nucleophilic adding of the thiol of reduced glutathione to a variety of electrophiles, in addition, GST binds with varying affinities a variety of hydrophobic compounds such as polycyclic aromatic hydrocarbons and other xenobiotics as pyrethroids etc. GST conjugates numerous xenobiotic with glutathione. Therefore the endogenous availability of glutathione at the target site might be a limiting factor for GST catalyzed conjugation and there by influence the protective function of the enzyme, but any alterations in the activity of GST can lead to alterations in activation-detoxification balance present in tissues to detoxify potential toxic agents (Rushmore and Pickett 1993). Significant decline was noticed in the activities of GSH-dependent antioxidant enzymes, GP\(_X\) and GST, in the
liver tissue of Group IV fishes compared to normal controls, reflecting an increased oxidative stress in λ cyhalothrin induced fishes. GPx offers protection to the cellular and sub cellular membranes from the peroxidative damage by eliminating hydrogen peroxide and lipid peroxidation. GST binds to many different lipophilic drugs; so it would be expected to bind λ cyhalothrin and act as an enzyme for GSH conjugation reactions. Inhibition of these enzymes may leads to the accumulation of these oxidants and makes liver cell membranes more susceptible to oxidative damage. GSH and GSH-dependent enzyme systems may be directly related to the pathogenic mechanisms of λ cyhalothrin induced fishes.

5.5.3 Antiperoxidative enzymes

Oxidative stress takes advantage of the available mitochondrial electron to make molecular oxygen, resulting in excess superoxide production in most tissues (Andreyev et al., 2005). These superoxide anions are converted to hydrogen peroxide and water with the help of a group of SOD (Firdovich 1995). Catalase, an enzyme that occurs in almost all aerobically respiring organisms, serves to protect cells from the toxic effects of hydrogen peroxide, by catalysing the hydrogen peroxide to water and oxygen (Moosavi et al., 1987).
Activities of antiperoxidative enzymes (SOD and CAT) were also significantly decreased in the liver tissue of \( \lambda \) cyhalothrin induced fishes in Group IV as compared to controls. Decrease in catalase activity could be due to flux of superoxide radicals, which have been reported to inhibit CAT activity (Pandey et al., 2001). Reduction in the activities of antiperoxidative enzymes in these fishes might be due to the increased generation of reactive oxygen radicals such as superoxide and hydrogen peroxide, which in turn leads to the inactivation of these enzyme activities in \( \lambda \) cyhalothrin exposed to \textit{O.mossambicus} showed a significant elevation in the level of lipid peroxidation along with a marked decline in the activities of superoxide dismutase and catalase, thus indicating the increased \( \lambda \) cyhalothrin induced oxidative stress condition. Inhibition of \( \lambda \) cyhalothrin on the liver tissue of \textit{O.mossambicus} may alter the antioxidant nature by the presence of free radicals.

**5.6 Changes in the levels of membrane-bound ATP\textsubscript{ase} activity and minerals in the liver tissue of \textit{Oreochromis mossambicus}**

A significant elevation was observed in the level of Ca\(^{2+}\) ions in Group IV fishes than compared with Group I control fishes. 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). \( \lambda \) cyhalothrin induced hepatotoxicity has been reported to enhance cyclase activity, resulting in increased formation of cAMP. (Subash et
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\textit{al.}, 1978). cAMP phosphorylates several sites on the C-terminal chains of the calcium channel and increases the probability of the calcium channel opening (Varadi \textit{et al.}, 1995). This may be the reason for enhanced activity of $\text{Ca}^{2+} \text{ATP}_{\text{ase}}$ and increased concentration of $\text{Ca}^{2+}$ observed in liver tissue of $\lambda$ cyhalothrin induced hepatotoxicity in fresh water fishes (Rekha and John Joseph 2011). Intracellular $\text{Ca}^{2+}$ 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 $\text{Ca}^{2+}$ accumulation may be involved in the dysfunction of the liver (Jan \textit{et al.}, 2005). Since $\text{Ca}^{+}$ and $\text{Na}^{+}$ 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 (Vincenzi 1971).

Significant elevation was observed in the level of $\text{Na}^{+}$ ions in Group I control fishes than compared with Group IV fishes. A significant elevation in the level of $\text{K}^{+}$ ions was found in Group IV fishes than compared with Group I control fishes.

The transport of $\text{Na}^{+}$ and $\text{K}^{+}$ intra and extra pools and the maintenance of the transmembrane gradients are important to cell function and integrity. The altered $\text{Na}^{+}/\text{K}^{+} \text{ATP}_{\text{ase}}$ dependent co-transport mechanism due to $\lambda$ cyhalothrin might have played an important role in the changes in the level of $\text{Na}^{+}/\text{K}^{+}$ imbalance and intracellular calcium overload (Lang 2007). $\lambda$ cyhalothrin treatment has probably
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altered in Ca^{2+} homeostasis by modulating the efflux of Ca^{2+} via the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (Ruhfus et al., 1996). In addition to this, the ability of \(\lambda\) cyhalothrin to maintain the integrity of membrane-bound ATP\textsubscript{ases} 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). \(\lambda\) cyhalothrin is also reported to de-normalize the content of potassium and calcium ion in piscian cells (Lang et al., 1998).

A significant decline was noticed in the activities of the membrane-bound ATP\textsubscript{ases} (Na\textsuperscript{+}, K\textsuperscript{+}, ATP\textsubscript{ase}, Mg\textsuperscript{2+} ATP\textsubscript{ase}, and Ca\textsuperscript{2+} ATP\textsubscript{ase}) in the liver tissue of Group IV fishes than compared with Group I control fishes. The Na\textsuperscript{+}/K\textsuperscript{+} ATP\textsubscript{ase} pump is responsible for the active transport of Na\textsuperscript{+} and K\textsuperscript{+} 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\textsuperscript{+}/K\textsuperscript{+} ATP\textsubscript{ase}. 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\textsuperscript{+}/K\textsuperscript{+} ATP\textsubscript{ase}, thereby leading to increased accumulation of Na\textsuperscript{+} ions in \(\lambda\) cyhalothrin exposed freshwater fishes (Rekha and John Joseph 2011). ATP\textsubscript{ases} are integral membrane protein which require 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 $\text{Mg}^{2+}$ ATP$_{ase}$, $\text{Ca}^{2+}$ ATP$_{ase}$, and $\text{Na}^+, \text{K}^+$ ATP$_{ase}$.

Activities of $\text{Na}^+, \text{K}^+$ ATP$_{ase}$, $\text{Mg}^{2+}$-ATP$_{ase}$ and $\text{Ca}^{2+}$ ATP$_{ase}$ in the liver were significantly lower in Group IV λ cyhalothrin induced toxic fishes, which is in line with previous reports (Sakaguchi et al., 1995). The intracellular concentration of calcium regulates the activity of the $\text{Mg}^{2+}$ and $\text{Na}^+, \text{K}^+$-ATP$_{ases}$. Bironaite and Ollinger (1997) have reported that lipid peroxidation can influence the function of $\text{Ca}^{2+}$ and $\text{Mg}^{2+}$-ATP$_{ases}$ and the activity of membrane $\text{Ca}^{2+}$-translocase. The marked loss in the activities of membrane-bound ATP$_{ases}$ 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 ATP$_{ase}$ activity and minerals in fresh water teleost fish $O.\text{mossambicus}$. It exerts cellular and sub cellular membrane destabilization in the liver by altering both non-enzymic and enzymic antioxidants (Rekha and John Joseph 2011).

5.7 Histopathology of the liver tissue of $O.\text{mossambicus}$

Light microscopic studies revealed that morphologic changes were more perceptible in the liver tissue of the exposed fish which was not noticed in the control
fish. On exposure to toxicants the liver tissue showed varying degree of degeneration of cell, hypertrophs of hepatocytes, fatty degeneration and vacuolization of cell cytoplasm, which were dose-dependent alterations. Liver is the detoxification area of toxicants. The organ most associated with the detoxification and biotransformation process is the liver and due to its function, position and blood supply, it is also one of the organs most affected by contaminants in the water (Camengo et al., 2007). Toxicity and histopathological studies are beneficial mechanisms to assess the pollution possibilities on exposure to toxicants. Therefore the significant pathological alterations in organs in contact with the pollutants appear to be a valuable biomarker of pollutant exposure and effect. Liver is also rich in proteins as it is the site of numerous metabolic activities. The decrease in protein content as observed in the present study in most of the fish tissues may be due to metabolic utilization of the ketoacids to gluconeogenesis pathway for the synthesis of glucose, or due to directing the free amino acids for the synthesis of proteins, or for the maintenance of osmo and ionic regulation (Schmidt Nielson, 1975).

It may be due to the generation of free radicals.

The liver of the studied fish showed vacuolar degeneration in the hepatocytes, focal area of necrosis, thrombosis formation in central vein, dilation and congestion in blood sinusoids and fibrosis. These changes may be attributed to direct toxic effects of pollutants on hepatocytes, since the liver is the site of detoxification of all types of toxins and chemicals (Soufy et al., 2007). The vacuolization of hepatocytes might
indicate an imbalance between the rate of synthesis of substances in the parenchymal cells and the rate of their release into the circulation system (Gingerich 1982). Oxygen deficiency, as a result of gill degeneration being the most common cause of the cellular degeneration in the liver (Eder et al., 1986). The vascular dilation, intravascular haemolysis and thrombosis formation observed in the blood vessels with subsequent stasis of blood may be also responsible for the cellular degeneration and necrosis in the liver (Mohamed 2001). The present results are in agreement with those observed by many authors who have studied the effects of different pollutants on fish liver (Mohamed 2001; Ptashynski et al., 2002; Fanta et al., 2003). Olojo et al., (2005) observed degeneration of the hepatocytes and focal necrosis in the liver of *Clarias gariepinus* exposed to lead. The same is the result we observed in our findings also. Exposure of *Oncorhynchus mykiss* to copper sulphate was found to induce degeneration of hepatocytes, sinusoidal dilation and congestion in the blood vessels of the liver (Atamanalp et al., 2008). Fatty accumulation is the characteristic feature of liver damage (Rouiller 1964).

5.8 Histopathology of the kidney tissue of *Oreochromis mossambicus*

The kidney is the vital organ of the body and the purpose of the kidney is to perpetuate homeostasis. It is not only involved in removal wastes from blood but it is also responsible for selective reabsorption, which helps in maintaining volume and pH
Discussion

of blood and body fluids and erythropoieses (Iqbal et al., 2004). The kidney is one of the first organs to be affected by contaminants in the water (Thophon et al., 2003). (Camergo and Martinez 2007) found cloudy swelling degeneration in the epithelium of renal tubules in the kidney of *P. lineatus* caged in Cambe stream, Brazil, polluted by industrial, domestic and agricultural wastes. These results are in agreement with the present study. The degeneration in the tubular cells suggests impairment in the reabsorption of electrolytes and infiltration process leading to imbalance in osmotic regulation of body fluids and changes in pH which in turn influences adversely various enzymatic processes in the fish. These pathological changes may be due to the preferential accumulation of pollutants that cause tubular degeneration leading to lymphocytic infiltration as a measure of resistance to the toxicants and tissue susceptibility (Huckabee and Blaylock 1972, Eaton 1974, Mount and Stephan 1967). These findings correlate with the present investigation.

5.9 Histopathology of the gill tissue of *Oreochromis mossambicus*

The gills, which participate in many important functions in the fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment and particularly sensitive to changes in the quality of the water are considered the primary target of the contaminants (Camergo and Martinez 2007; Fernandes and Mazon 2003). The observed alterations like proliferation of the
epithelial cells, partial fusion of some secondary lamellae and epithelial lifting are defense mechanisms, since, in general, these results are in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants (Fernandes and Mazon 2003; Poleksic and Mitrovic 1994). The cellular damage observed in the gills in terms of epithelial proliferation, separation of the epithelial layer from supportive tissues and necrosis can adversely affect the gas exchange and ionic regulation (Dutta et al., 1993). The observed edematous changes in gill filaments and secondary lamellae probably due to increased capillary permeability (Olurin et. al., 2006). The present results are in agreement with those observed in other fish species under the influence of different pollutants (Kakuta and Murachi 1997; Olurin et al., 2006). In this respect, (Camergo and Martinez 2007) observed hyperplasia of the epithelial cells, fusion of secondary lamellae, lifting of the lamellar epithelium and blood congestion in the gills of P.lineatus caged in Cambe stream, Brazil, polluted by industrial, domestic and agricultural wastes.
5.10 Histopathology of the brain tissue of *Oreochromis mossambicus*

Brain has been used as the key organ to understand the toxic impact of λ cyhalothrin in *O. mossambicus*. Brain is the centre of reasoning, regulation, translation and coordination and is aided by glutagenic and cholinergic nerve (Mastan and Shaffi 2010). Changes associated with the brain to sub lethal exposure to λ cyhalothrin are not reported in the available literature. The present experimental trials revealed that λ cyhalothrin may also be neurotoxic, evidenced by the histological changes. These findings agreed with earlier reports by Omitoyin *et al.*, 2006 in *Clarias gariepinus* exposed to glyphosate.