5. 1. INTRODUCTION

Fish are sensitive organisms and show a wide range of responses to changes in their aquatic habitat. Pesticides as environmental contaminants not only at lethal concentrations but also in a low level, have profound effect on aquatic organisms. It is possible to evaluate the potential of pesticides through histopathological studies because trace levels of pesticides, which do not cause animal mortality may cause considerable damage to the organism. Histopathology is the study of diseases and disease processes by microscopical observations of thin sections of preserved and stained tissues. It is one of the effective tools for ecotoxicology and risk assessments. The impact of a pollutant on a fish can be assessed by examining the different tissues for morphological alterations. Histology can identify signs of disease which are not easily found on gross examination. These changes have been widely used as biomarkers in determining the health status of fish exposed to pollutants both in the laboratory (Wester and Canton, 1991; Thophon et al., 2003) and field studies (Teh et al., 1997; Sulekha and Mercy, 2009). According to Bernet et al. (1999), histopathology is a rapid method to detect the effects of contaminants, especially chronic ones, in various tissues and organs.

Considerable interest has been shown in histopathological study of fish exposed to sublethal concentrations of contaminants in recent years. Using histopathological observations, one can evaluate the changes in specific target organs like gills, kidney and liver which are doing vital functions such as respiration, excretion, accumulation and biotransformation of xenobiotics in the fish (Gemhofer et al., 2001) and serve as warning signs of the detrimental
effect caused to animal health (Hinton and Lauren, 1993). The changes in these vital organs are easier to identify than functional ones. Histological changes in tissues provide adequate information regarding the functional response of organisms to the toxicants. One of the early symptoms of pesticide toxicity is respiratory distress. Histological alterations in fish gill serve as warning system for water quality assessment in sublethal and chronic situations. Fishes have to pass large amount of water for the purpose of respiration and gills being delicate structures get affected when exposed to the pesticides dissolved in water. It is a multifunctional organ of the fish that performs gaseous exchange, osmoregulation and excretion. Gill epithelium provides an extensive surface of contact with the environment to facilitate these functions. In fish the area of gill epithelium is almost equal to the area of the skin and is very thin to allow exchange of gas to regulate the exchange of salts and water and excrete nitrogenous waste. Any structural damage of gills caused by a toxicant affects the osmoregulaory as well as respiratory functions.

Liver performs several functions such as synthesis of components of blood plasma, glycogen storage and release of glucose to the blood. It is the organ most associated with the detoxification process due to its position, blood supply and functions, hence one of the organs mainly affected by the contaminants in the water (Camargo and Martinez, 2007). Liver can store more toxicants than any other tissues in the body. Reports indicate that liver is the organ with the highest concentration of the pesticide and the greatest damage is caused to it during pesticide treatment (Nagabhushanam et al., 1994). In teleost fish, kidney is a mixed organ consisting of
reticuloendothelial, haemopoietic, endocrine and excretory elements. Ammonia, urea and creatinin are the nitrogen containing waste products from the metabolism, excreted by the kidney. It is a vital structure involved in the maintenance of homeostasis, removal of wastes from blood, sensitive reabsorption to maintain pH and volumes of blood and body fluids and erythropoieses. Contaminants like pesticides may bring histological changes in the tubular epithelium and glomerulus (Teh et al., 1997). Muscles of teleosts are in the form of red and white muscles depending on the vascular and metabolic differences. Myotomes consist of bundles of white muscle fibres bound together by connective tissue. Pesticide pollution affects muscular coordination that leads to abnormal swimming behaviour in fish.

The basic components, of a teleost brain is similar to the brain of higher animals with some differences in form and complexity. The two types of tissues in the brain, the nervous tissue proper and the connective tissue which is supporting it show different types of responses. The pathological changes associated with neural dysfunction are usually observed in meninges, blood vessels and microglia. In fish, the intestine is a simple tube which does not form a colon posteriorly. It is primarily involved in the vital function of absorption of digested food material. Pesticides reach the intestine along with food and water and induce structural and functional abnormalities. Since fishes are one of the major victim of aquatic pollution, pesticides interfere with their reproductive process also. Testes are paired organs, suspended by mesenteries from the dorsal abdominal wall. A number of seminiferous tubules separated from one another by a very thin layer of interstitial tissue. These tubules are packed together and contain cells at different stages of
spermatogenesis. Ovaries are also suspended from the abdominal wall by a mesentery in the female fish and they appear as a small cluster of minute spheres in the immature one. Pesticides accumulate in gonads and cause pathological changes.

Much attention has been paid to histopathological investigations, while conducting chronic tests in fish exposed to several pesticides, but only a few studies have been reported so far in fish treated with synthetic pyrethroids and neonicotinoids. Although the pesticides under study impair the haematological and biochemical components of the fish, these physiological studies alone are not sufficient for the complete understanding of pathological conditions of fish under pollutant stress. It is also essential to analyse the changes in histology of the pesticide treated fish. Therefore, it was decided to study the histopathologic effects on gill, liver, kidney, muscle, brain, intestine and gonads of *Cyprinus carpio* exposed to lambda-cyhalothrin and thiamethoxam.

### 5.2. REVIEW OF LITERATURE

Literature abounds regarding pesticide induced changes in various tissues of several fish species, but scanty information is available in common carp in this regard. Some of the histopathological studies using different pesticides in freshwater fish including *C. carpio* have been reported here.

The freshwater teleost *Puntius ticto* exposed to BHC, lindane, endosulfan was used to find histopathological changes in gill structure. Inflammatory reaction and complete dystrophy of the lamellar structure of gills for BHC; disruption of the epithelial covering of gills for lindane and
shrinkage of gill lamellae for endosulfan exposure were noticed (Singh and Sahai, 1990). Disorganisation of serosa and mucosal folds, swelling, vacuolation and pycnosis of epithelium of digestive tissues of *Anabas testudineus* treated with lindane and atropine sulfate was documented by Bakthavathsalam and Rajaratnam (1990). Disappearance of epithelium at the tip of villi of the intestine of the gobid fish *Glossogobius giuris* affected by malathion treatment was recorded by Dhanalakshmi, (1991). Malathion induced changes in the gills such as inflammatory alterations of lamellar epithelium and hyperplasia for short term exposure and proliferative loss of epithelium for long term exposure have been observed in *Cirrhinus mrigala* (Roy *et al.*, 1991). Histopathological changes of liver tissue were studied in *Heteropneustes fossilis* subjected to acute thiodon toxicity showing extensive degeneration of cytoplasm with pyknosis of nuclei (Narayan and Singh, 1991); in *Tilapia mossambicus* subjected to fenvalerate showing irregular shaped cells and displacement of cells (Radhaiah and Rao, 1992) and in *L. rohita* subjected to phosphamidon showing nuclear enlargement, vacuolation and necrosis (Medda *et al.*, 1992).

Sahai, (1992) studied histopathological changes in the testis of *P. ticto* exposed to BHC, lindane, endosulfan and malathion. He has observed testicular degeneration, atrophy, pycnosis, rupture and disortion of tubules in the affected fish. Dutta *et al.* (1993) showed necrosis and atrophy of liver in *H. fossilis* as toxic effects of malathion. Histological changes in the liver of *Noemacheilous aureus* exposed to cypermethrin at lethal and sublethal concentrations were reported by Jabde and RaiNat (1993). Severe histopathological changes were induced in *C. carpio* subjected to acute and
subacute toxicity of atrazine (Neskovic et al., 1993). Pathological effects of gut of *C. carp* exposed to dimethoate included erosion in muscosal folds, vacuole formation, enlargement of the mucous cells and spaces in the submucosa (Tembhre and Kumar, 1994). This fish when treated with malathion and Sevin showed hypertrophy of renal cells, formation of vacuoles, necrosis and degeneration of renal components in the kidney (Dhanapakiam and Premalatha, 1994). A report by Jain and Mishra (1994) on atrazine impact on liver of *P. ticto* showed pathological lesions such as infiltration of blood cells, hypertrophy and vacuolar degeneration of hepatocytes. Hepatopencreas and gills of chlorpyrifos exposed *Tilapia zillii* were observed by Sherif and Eisa (1994). Dilation of the blood vessels, tilangetasis in the secondary lamellae in gills and vacuolar degeneration and necrosis of hepatocytes were documented.

Investigation on histopathological changes in the seminal vesicle of *G. giuris* (Ham) treated with fenthion was carried out by Zutshi et al. (1995). Disorganized secretory epithelial cells during non-breeding period and thick interlobular connective tissue, necrotic sperms in the lobules, degeneration of secretory cells were well observed. Histopathological effects of monocrotophos and fenvalerate in the gill of *L. rohita* were reported by Vijayalakshmi and Tilak (1996). Necrotic changes and alterations in the shape of filaments caused by monocrotophos; atrophy in gill filaments by fenvalerate; fusion and atrophy of secondary gill lamellae due to mixtures of monocrotophos and fenvalerate were reported. Impact of carbaryl on *Nardus nardus* was assessed by the histopathological examination of intestine showing broken serosa, fringed longitudinal muscles, necrosis in intestinal
villi and reduced tunica propria (Suda and Asha, 1999). Effects of trifluralin and severe changes on gills and kidney structure have been studied by Vesna and Vesela (1999).

A loss in cellular integrity, cells without nucleus and hypertrophy were noticed in the liver of *H. fossilis* on its long term exposure to acenphthene (Dwivedi, 2000). Santhakumar *et al.* (2001) studied the impact of monocrotophos on *A. testudineus* and recorded degeneration and necrosis of epithelial cells, distortion of the secondary lamellae in gills. Rodrigues *et al.* (2001) documented lesions in the liver of *Prochilodus lineatus* after its exposure to sublethal concentrations of trichlorfon. Wannee *et al.* (2002) used *Oreochromis niloticus*, to study the effects of roundup, a glyphosate herbicide. Vacuolation of hepatocytes and nuclear pycnosis in the liver; dialation of Bowman’s space and accumulation of hyaline droplets in the tubular epithelial cells in kidney and filament cell proliferation, lamellar cell hyperplasia, lamellar fusion, epithelial lifting and aneurysm in gills were detected in the treated fish.

Exposure of *Gambusia affinis* to sublethal concentrations of malathion caused necrosis, desquamation of secondary lamellar epithelium, lifting up of epithelium, intraepithelial edema, hypertrophy and hyperplasia of epithelial cells (Cengiz and Unlu, 2003) and to monocrotophos caused morphological aberrations and deformities in the primary and secondary lamellae (Rao *et al.*, 2005) of the gills. Chlorpyrifos induced changes were noticed in vital tissues of gills, liver, brain and kidney in *Catla catla* (Tilak *et al.*, 2005). The extent of damage caused to liver by carbofuran and cypermethrin was analysed by Sarkar *et al.* (2005) in *L. rohita*. Hyperplasia, disintegration of hepatic mass
and focal coagulative necrosis were the observed changes. *Cyprinus carpio* exposed to deltamethrin was characterized by desquamation, necrosis, aneurism, edema, epithelial lifting, hyperplasia and fusion of secondary lamellae in gills, and pycnotic nuclei in the haematopoietic tissue, degeneration of glomerulus, intracytoplasmic vacuoles in epithelial cells of renal tubules in kidney (Cengiz, 2006), to monocrotophos showed upliftment of epithelial cells, hyperplasia and decrease in the density of mucous cells in the gills (Johal *et al.*, 2007); and to endosulfan produced hyperplasia, lamellar fusion, curling and bulging of tips of primary gill lamellae (Riji and Jayabalan, 2007). Endosulfan treatment of *Oncorhynchus mykiss* resulted in lesions in liver, spleen, kidney and gills as documented by Altinok and Capkin. (2007). Cytotoxicity of neonicotinoid insecticide imidacloprid was studied in gill cell line of flounder *Paralichthys olivaceous* (Su Feng *et al.*, 2007) revealed that the nuclei remained normal but mitochondria were severely damaged, swollen or disrupted. Velmurugan *et al.* (2007) determined histopathological alterations in gills, kidney, liver and intestine of *C. mrigala* treated with lambda- cyhalothrin and fenvalerate. Epithelial hyperplasia, necrosis, desquamation, lamellar fusion, epithelial lifting, edema, swelling and curling of secondary lamellae in gills; necrosis, pycnotic nuclei and hypertrophied epithelial cells in kidney; congestion, cloudy swelling of hepatocytes in liver and necrosis, atrophy of epithelial cells, infiltration of lymphocytes into lamina propria in intestine were the observed changes.

Impact of Butachlor and Machete on *Channa punctatus* studied by Tilak *et al.* (2007), showed severe necrosis, cloudy swelling, cellular hypertrophy, granular cytoplasm, vacuole formation and decrease in the size
of distal convoluted tubules of kidney. Jeyachandran and Pugazhendy (2009) have evaluated the effects of atrazine on the gills of *L. rohita* fingerlings and revealed that the herbicide caused epithelial hyperplasia, curling of secondary lamellae, changes in chloride cells, pycnotic nuclei and vacuolization. Dichlorvos caused hyperplasia desquamation, necrosis of epithelium, edema, lamellar fusion, curling of secondary lamellae in gills and cloudy swelling of hepatocytes, congestion, vacuolar degeneration, karyolysis, dilation of sinusoids and nuclear hypertrophy in liver in *C. mrigala* and cypermethrin induced lesions in gills, liver and kidney in *Clarias gariepinus* exposed to sublethal concentrations (Velmurugan *et al.*, 2009.a,b.).

Synthetic pyrethroids like bifenthrin has produced degeneration of hepatocytes in *O. mykiss* (Velisek *et al.*, 2009) and cyfluthrin has induced histopathological and behavioural changes in *C. carpio* (Aylin *et al.*, 2009). Indirabai *et al.* (2010) assessed subacute effects on endosulfan on *L. rohita* and found pathological lesions leading to necrosis of hepatocytes, glomerulus of kidney and gill filaments. Over secretion of mucous, hyperplasia and fusion of gill lamellae, epithelial uplifting and necrosis of gill epithelial cells were the alterations observed in *H. fossilis* treated with azadirachtin (Kumar *et al.*, 2010). Lamellar hyperplasia in gill, inflammation and focal necrosis in liver and kidney were caused by Maneb and carbaryl in *O. mykiss* (Boran *et al.*, 2010). *C. mykiss* exposed to carbosulfan, propineb and benomyl showed cell necrosis, degeneration and edema in liver, kidney and spleen as well as lamellar fusion, hyperplasia, epithelia lifting, necrosis, hypertrophy and sloughing of epithelium (Capkin *et al.*, 2010). The effect of edifenphos on *O. niloticus* was assessed by Gaafar *et al.* (2010) and various degrees of
pathological lesions in gills, hepatopancreas, spleen, kidney, brain and other organs have been recorded.

Histopathological alterations of freshwater fishes exposed to acute and sublethal toxicity of pesticides were reported by Joseph and Raj, (2011). Malathion induced changes like dissociation of hepatocytes, broken sinusoidal endothelium vacuoles in hepatocytes; degeneration of kidney tubules and haematopoietic cells, necrosis and haemorrhage as well as affected oocytes were reported by Deka and Mahanta, (2012) in *Heteropneustes fossilis*. Synthetic pyrethroid caused vacuolation, necrosis and degeneration of hepatocytes; bulging, distortion of filaments and hyperplasia of epithelium and vacuolation and breakdown of muscle fibres in *Catla catla* (Muthuviveganandavel et al. 2013). Hypertrophy, vacuolation, degeneration of hepatocytes and dilation on sinusoids in liver of deltamethrin treated *Xiphophorus helleri* was documented by Yon et al. (2014). Destruction of gill lamella, epithelial hyperplasia and epithelial hypertrophy in gills and vacuolation, fatty degeneration and hypertrophy of hepatocytes were reported in *Clarias gariepinus* treated with cypermethrin (Ojutiku et al. 2014). Toxicity of chlorfos caused abnormal lamellae, marginal dilation, hyperplasia of epithelial cells and gill deformation as well as necrosis, focal infiltration of lymphocytes, hepatocyte degeneration of liver (Al-Mamoori et al., 2014); cyhalothrin caused steatosis dystrophy of liver (Richterova et al., 2014) and fenthion caused swelling and necrosis of liver, necrosis and distended tubules of kidney, primary lamellar fusion, swollen, deformed, hyperplasia of secondary lamellae and ruptured intestinal villi, enlarged mucous cells and proliferation of mucous cells of intestine (Leena, 2014) in *Cyprinus carpio*. 
Literature reviewed so far has shown that though much works have been undertaken in histopathology to evaluate the toxicity of pesticides to fish, only a few reports were available for synthetic pyrethroid and neonicotinoid toxicity.

5.3. Materials and Methods

*Cyprinus carpio* L. exposed to sublethal concentrations (1/50\textsuperscript{th}, 1/20\textsuperscript{th}, 1/10\textsuperscript{th} and 1/5\textsuperscript{th} of LC\textsubscript{50} values) of lambda-cyhalothrin and thiamethoxam for 120 days were subjected to histopathological studies. Fish reared in pesticide free water was used as control. Tissues namely gills, liver, kidney, intestine, muscle, brain, testis and ovary of the treated as well as control fish were dissected out and fixed in Bouin’s fluid (Humason, 1972). After fixation for 24 hours, small fractions of the tissues were dehydrated, embedded in paraffin and sections were cut. These sections were stained in Harris haematoxalyn and Eosin and microphotographs were taken using Uno Med Binocular Microscope (PZRM - 700).

5.4. Results

5.4.1. Gill

The gill of the control fish was shown in Plate – 2. a. The gill filaments of primary lamellae were flattened extensions running at right angles to the branchial arches in double rows. Each filament consisted of dorsal and ventral rows of secondary lamellae. Secondary lamellae were simple and thinner than primary lamellae and were lined by a squamous epithelium below which were blood sinuses separated by pillar cells. Between the secondary lamellae, the primary lamella was lined by a thick stratified epithelium, which contained mucous and chloride cells.
Histopathological observations of lambda- cyhalothrin treated *C. carpio* at concentration 0.021 $\mu$g/l showed lamellar bending, lamellar fusion, hyperplasia of filaments and interlamellar epithelium. Significant changes in the fish exposed to 0.053 $\mu$g/l lambda- cyhalothrin were edema, necrosis and haemorrhage. Fish exposed to 0.106 $\mu$g/l concentration showed edematous separation of epithelial cells from the pillar cells, epithelial necrosis, hyperplasia of filaments and interlamellar epithelium. Severe damage to the gill filaments were found with the loss of secondary gill lamellae, epithelial desquamation and enlargement of primary gill lamellae at 0.212 $\mu$g/l concentration (Plate-2. b-e).

Fish exposed to 2.565 mg/l thiamethoxam revealed changes like epithelial necrosis, lamellar bending and lamellar fusion. At 6.413 mg/l concentration, lamellar bending, lamellar fusion and hyperplasia of filaments and interlamellar epithelium were noticed. These changes became more apparent at 12.826 mg/l dosage. Disfigurement of the secondary and primary gill filaments were seen in 25.651 mg/l thiamethoxam treated fish (Plate-3. a-d).

### 5.4.2. Liver

Liver of the fish from the control composed of hexagonal hepatic cells arranged in cords around large blood sinusoids. Hepatocytes had distinct central nuclei and homogenous cytoplasm. Exocrine pancreas was within the hepatic tissue in diffused condition (Plate- 4. a).

Fish exposed to 0.021 $\mu$g/l lambda- cyhalothrin revealed changes like edema, hypertrophy of hepatocytes, necrosis and haemorrhage. At 0.053 $\mu$g/l concentration, the liver cells were distended by clear lipid vacuoles. Edema and necrotic zones were also seen. Fatty vacuolization of hepatic cells with infiltration of macrophages and
lymphocytes were the significant changes in 0.106 µg/l lambda-cyhalothrin treated carp. Degenerative changes and disorganization of hepatic cells as well as derangement of pancreatic acini were observed at 0.212 µg/l (Plate- 4. b-e).

Histological alterations found in thiamethoxam treated *C. carpio* at the lowest concentration (2.565 mg/l) were congestion at certain regions, haemorrhage, vacuolization of hepatocytes and edema. These changes were apparent at 6.413 mg/l dosage. Significant changes at 12.826 mg/l concentration included phagocytic invasion, haemorrhage and hepatocellular degeneration. Highest dose (25.651 mg/l) caused degeneration and disorganization of hepatocytes (Plate-5. a-d).

**5.4.3. Kidney**

Section through the kidney of control fish (Plate-6. a) shows that the tissue comprised of uriniferous tubules and interstitial haemopoietic tissue. Uriniferous tissue consisted of glomerulus which was made up of capillaries and surrounded by Bowman’s capsule with a short neck followed by coiled tubule.

Plate-6. b-e shows histological abnormalities caused by lambda-cyhalothrin to the kidney of *C. carpio*. At 0.021 µg/l concentration, edema, proliferation of leucocytes and haemorrhage were observed. Tubular necrosis, interstitial edema and atrophy of interrenal tissue were the changes in 0.053 µg/l dose. Fish treated with 0.106 µg/l concentration showed interstitial edema and atrophy, collapse of glomeruli and cloudy swelling of tubules. At 0.212 µg/l exposure, interstitial edema, tubular necrosis, collapse of glomeruli and proliferation of leucocytes were prominent.

Thiamethoxam treatment (2.565 mg/l) resulted in glomerular swelling and edematous interstitial tissue in the kidney of carp. Cloudy swelling of glomerulus,
tubular necrosis interstitial edema and leucocyte aggregation were noticed at 6.413 mg/l concentration. Fish exposed to 12.826 mg/l thiamethoxam revealed changes like haemorrhage, tubular necrosis, proliferation of leucocytes and glomerular swelling. Focal cloudy swelling, tubular necrosis, haemorrhage and atrophy of interrenal tissue were found at 25.651 mg/l dose (Plate-7. a-d).

5.4.4. Intestine

Control fish intestine (Plate- 8. a) was a simple tube, the wall of which comprised of inner mucosa, middle sub mucosa and an outer muscularis mucosal layer. The mucosa was seen folded into number of finger like processes, the microvilli which project into the lumen of the intestine. The epithelial lining of mucosa consisted of absorptive cells. Sub mucosa contained connective tissue fibers, blood vessels and nerve endings. Muscularis was well developed and embedded in loose connective tissue richly supplied with blood capillaries. Muscularis consisted of smooth muscle fibers arranged as the outer longitudinal and inner circular.

Lambda- cyhalothrin (0.021 µg/l ) exposed fish showed haemorrhage and necrosis of intestinal epithelium at some places. These changes were well noticed along with edema, vacuolization of mucous epithelium and accumulation of macrophages at 0.053 µg/l concentration. At 0.106 µg/l dose, necrosis and sloughing of the mucosal layer, edematous zones, vacuolization, and accumulation of macrophages, atrophy and desquamation of intestinal epithelium were observed. These changes were more pronounced at 0.212 µg/l concentration (Plate- 8. b-e).

Plate-9. a-d revealed histopathological changes of the intestine of thiamethoxam treated fish. Necrosis in epithelial lining and edema in sub mucosal layer were apparent at the lowest dose (2.565 mg/l). Edema, massive necrosis,
haemorrhage and ulceration were found in 6.413 mg/l concentration. These changes were more pronounced in intestine of carp treated with 12.826 mg/l pesticide. Sloughing of mucosal layer, degenerative changes in epithelial lining, atrophy and infiltration of macrophages in the sub mucosal layer were the observed alterations at the highest concentration (25.651 mg/l) of thiamethoxam.

5.4.5. Muscle

Plate-10. a shows muscle of the control fish. The presence of normal myotomes, with equally spaced bundles of muscle fibres bound together by connective tissue. Each muscle fibre was surrounded by endomysium.

C. carpio exposed to lambda-cyhalothrin at 0.021 µg/l concentration showed inflammation, myofibrillar necrosis and marked variation in fibre size. Histopathological changes at 0.053 µg/l dose were intramuscular edema, thickening and separation of muscle bundles and focal necrosis. Edema, degenerative changes, macrophage infiltration were found in 0.106 µg/l exposure. In fish treated with 0.212 µg/l dosage necrosis, myodegeneration and infiltration by macrophages were observed (Plate-10. b-e).

In thiamethoxam treated carp at 2.565 mg/l dose intramuscular edema, thickening and separation of muscle bundles and necrosis were seen. In fish exposed to 6.413 mg/l thiamethoxam intramuscular edema and necrosis were noticed. Muscular atrophy, separation of muscle bundles and macrophage infiltration were the prominent features at 12.826 mg/l dosage. In fish treated with 25.651 mg/l concentration, necrosis, edema, atrophy and macrophage infiltration were observed (Plate-11. a-d).
5.4.6. Brain

Brain of the control fish is shown in Plate- 12 a. Brain consisted of two types of tissue, the nervous tissue and the connective tissue. Highly specialized nervous tissue proper comprised of neurons and the neuroglia. The nervous tissue proper was supported by the connective tissue which comprised the meninges and it supplied the blood vessels and lymphatics.

Plate- 12. b-e revealed histopathological alterations in the brain of *C. carpio* treated with lambda- cyhalothrin. At 0.021 µg/l concentration, edematous zones, vacuolation and infiltration by macrophages were found. Severe edema and macrophage infiltration were observed at 0.053 µg/l dosage. Exposure to 0.106 µg/l concentration resulted in edema of cerebellum, prominent purkinjee layer and degenerative changes. Edema of cerebellum, reactive gliosis, vacuolation and infiltration of macrophages were the changes seen at 0.212 µg/l dosage.

Thiamethoxam treatment of the fish at 2.565 mg/l concentration brought edematous zones and vacuolation in the brain. These changes were well noticed in fish brain at 6.413 mg/l dose. At 12.826 mg/l thiamethoxam, edema, vacuolation, degenerative changes and macrophage infiltration were observed. Focal edema, vacuolation, degeneration of neural cells and macrophage infiltration were prominent at 25.651 mg/l dosage (Plate - 13. a-d).

5.4.7. Testis

Testis composed of a number of seminiferous tubules of varying sizes. They were packed closely and were separated from one another by a very thin layer of interstitial tissue. Spermatogonic epithelium lined the tubules internally which gave
rise to spermatocytes. Spermatocytes developed into spermatids which then transformed into spermatozoa. The glandular tissue in the central portion of the testis consisted of interstitial glandular cells, fibroblasts, blood vessels and lymph vessels (Plate- 14. a).

Histological alterations of the testis in lambda-cyhalothrin (0.021 µg/l) treated fish showed vacuolation, condensation of tubular cells, increased interlobular areas, infiltration of leucocytes. Exposure to 0.053 µg/l concentration also resulted in similar changes in testis. In addition to these changes disintegration of basement membrane as well as regression of tubule were noticed in 0.106 µg/l dosage. At 0.212 µg/l regressed tubule, disintegrated basement membrane, diffused tubule, disintegrated tubule and further increase in intertubular space were observed (Plate-14. b-e).

Thiamethoxam treatment given to C. carpio resulted in histopathological changes in testis and were shown in Plate- 15 a-d. Vacuoles and increased interlobular areas were observed at 2.565 mg/l. Necrosis and foam cells in the lobular lumen were the significant changes at 6.413 mg/l dose. Exposure to 12.826 mg/l thiamethoxam resulted in condensation of tubular cells, disintegrated basement membrane, diffused tubules and increased interlobular areas. These changes were more pronounced at 25.651 mg/l concentration.

5.4.8. Ovary

The ovarian wall was differentiated into an outer peritoneum, tunica albuginea containing connective tissue, muscle fibres and blood capillaries as well as the innermost layer the germinal epithelium. Ovary contained different stages of oocytes and intact ovigerous lamellae and follicular lining. Immature oocytes were of small
sized with large nuclei and deeply stained cytoplasm whereas maturing oocytes contained large nuclei, poorly stained cytoplasm and yolk globules (Plate- 16. a).

Histopathological observations of C. carpio treated with lambda- cyhalothrin at 0.021 µg/l concentration revealed changes like necrosis, nuclear retraction, presence of pre-ovulatory atretic follicles and vacuolation in cytoplasm of oocytes. Fish exposed to 0.053 µg/l dosage showed nuclear retraction, atretic follicles, increased interfollicular space, degeneration of pre vitellogenic and vitellogenic oocytes. Decrease in size of oocytes, empty follicles with necrosis of nuclei and expelled nuclei, increased atresia, infiltration of macrophages and large interfollicular space were the alterations found in ovary of fish treated with 0.106 µg/l dosage. At 0.212 µg/l concentration atresia, empty follicles with expelled nuclei, increased interfollicular space and increased degeneration of oocytes predominated (Plate- 16. b- e).

Plate- 17. a- d revealed histological changes in ovary of the fish exposed to thiamethoxam. At 2.565 mg/l dose atresia, presence of empty follicles with expelled nuclei, nuclear retraction, damaged ovigerous lamellae and increased interfollicular space were noticed. Nuclear retraction, atresia and the presence of free floating follicular lining were the observed changes in ovary at 6.413 mg/l concentration. Fish exposed to 12.826 mg/l dose showed atresia, empty follicles, disruption and vacuolation in cytoplasm and reduction in size of oocytes. Large interfollicular space, empty follicles, nuclear retraction, macrophage infiltration and reduced size of oocytes were observed in ovary of fish treated with 25.651 mg/l thiamethoxam.
5. 5. Discussion

Histological preparations in the evaluation of effects of lambda-cyhalothrin and thiamethoxam on *C. carpio* form an essential part of this investigation because they describe tissue pathology caused by the pesticides. The present work proves the toxic potential of the pesticides and showed moderate to severe histopathological alterations in various tissues depending upon the doses, which could lead to metabolic changes in the fish.

In this study fish exposed to lower (1/50th and 1/20th of LC50) concentrations of lambda-cyhalothrin revealed alterations in the gills such as lamellar bending, lamellar fusion, hyperplasia of filaments and inter lamellar epithelium, edema and necrosis. Similar changes were also noticed in thiamethoxam treated fish at these concentrations. Since gills got exposed more to the pesticides in water and were the primary route of entry for the toxic chemical, hyperplasia and edema of gill lamellae as well as increased mucus production acted as defense mechanisms to prevent their contact with the toxicant in the water medium. These results were in agreement with the histopathological studies conducted to show the toxic impacts of malathion on *Cirrhinus mrigala* (Roy and Datta, 1991); trifluralin on carp (Vesna and Vasela, 1999); organochlorines and organophosphates on *C. carpio* (Das and Mukherjee, 2000); diazinon on *Lepomis macrochirus* (Dutta *et al.*, 1997); monocrotophos on *Anabas testudineus* (Santhakumar *et al.*, 2001); deltamethrin on common carp (Cengiz, 2006); atrazine on *Labeo rohita* (Jeyachandran and Pugazhendy, 2009); azadirachtin on *Heteropneustes fossilis* (Kumar *et al.*, 2010); gammalin 20 on *Clarias gariepinus* (Lawrence and Ogbomida, 2010). Lamellar fusion diminished the vulnerable gill surface area (Mallatt, 1985) and the epithelium when lifted up, increased the distance between the pesticide and blood stream. Epithelial proliferation
of secondary gill lamellae resulted as a response of the malpighian cells to chemical irritation. These cells migrated distally and accumulated at the edge of the secondary lamella which led to the formation of lamellar fusion and terminal lamellar clubbing (Robert, 2001).

*C. carpio* treated with higher (1/10\textsuperscript{th} and 1/5\textsuperscript{th} of LC\textsubscript{50}) concentrations of lambda-cyhalothrin showed severe damage to gill filaments, loss of secondary lamellae and epithelial desquamation. Damage to the gill filaments were also observed in fish exposed to thiamethoxam at these concentrations. However lambda-cyhalothrin caused severe injury to the gills of the fish when compared to thiamethoxam. The histological alterations in the gills were indicative of diminished oxygen supply to the fish resulting in hypoxic condition. Pesticides broke down the adhesion between epithelial cells and the underlying pillar cells, which was accompanied by a collapse of the structural integrity of the secondary lamellae and subsequent failure of the respiratory functioning of the gills (Skidmore and Tovell, 1972). The results were in conformity with other investigations which had documented similar changes in the gills of several fish species treated with various toxicants (Kakuta and Murachi, 1997; De Silva and Samayawardhena, 2002; Rao et al., 2005 and Pragna et al., 2010).

Although not lethal, gill damage caused by pesticides is important from the aspect of morbidity as it retards growth and affects reproduction (Das and Mukherjee, 2000.b.). Gills are not only involved in gaseous exchange, but also in maintaining acid-base balance, ionic transport and excretion of nitrogenous wastes. Therefore, injury to the gills would affect the whole body of fish and its health.
Liver is the organ doing detoxification process and because of its position, blood supply and its function, like gills liver also one of the organs, most affected by pesticides in water. *C. carpio* treated with lower concentrations of lambda-cyhalothrin showed edema, hypertrophy of hepatocytes, necrosis, haemorrhage and lipid vacuolization of hepatic cells. These alterations were also observed in thiamethoxam treated fish at lower concentrations. At higher doses of thiamethoxam, degenerative changes and disorganization of hepatocytes and phagocytic invasion were apparent and these changes were found to be intensified in the liver of lambda-cyhalothrin treated fish. Histopathological alterations in liver may be attributed to the direct toxic effect of pesticides on hepatocytes because it is the center of pesticide metabolism and it accumulated the toxic chemical.

Similar observations were made in different fishes induced by various pesticides. Phosphomidon induced changes in liver of *Labeo rohita* fingerlings including vacuolation of cells with increase in cell size, hepatic cord disarray and derangement of pancreatic acini (Medda *et al.*, 1992). Increasing hepatocyte size with pyknotic nuclei, infiltration of leucocytes, hydropic degeneration, cloudy swelling, focal necrosis and presence of vacuoles were reported in glyphosate induced *Oreochromis niloticus* (Wannee *et al.*, 2002). Vacuolar degeneration, swelling in the hepatocytes with indistinguishable cellular outline were found in *Oreochromis mossambicus* treated with dimethoate (Pragna *et al.*, 2010). *Labeo rohita* exposed to cypermethrin showed hyperplasia, vacuolation, disrupted hepatocytes, focal necrosis and disorganised hepatic canaliculi (Sarkar *et al.*, 2005). Deltamethrin induced changes in *Gambusia affinis* included hypertrophy of hepatocytes, focal necrosis and fatty degeneration (Cengiz and Unlu, 2006).
In the present study lipid vacuoles appeared as clear vesicles occupying the whole cytoplasm of the liver of pesticide treated fish. Biochemical changes in liver profile showing the increased lipid content during exposure is related to the hepatocyte damage of liver. Lipid accumulation (hepatic steatosis) induced by gamma hexachlorocyclohexane in *Brachydanio rerio* was reported by Braunbeck *et al.* (1990) who stated that lipid vesicles were the morphological expression of a blockage in the metabolism of hepatic triglycerides. According to Gingerich, (1982) 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 systemic circulation. The toxicant absorbed is transported by blood to the liver for detoxification and if detoxified it may be excreted through bile or through the blood stream reaches kidney or gill for excretion (Lindstoma- Seppa *et al.*, 1981). Histopathological alterations in liver led to reduction in its functional efficiency and therefore malfunctioning of several organ systems of the fish including gill and kidney.

*C. carpio* exposed to lambda-cyhalothrin at lower concentrations (0.021 µg/l, 0.053 µg/l) showed histological abnormalities in the kidney such as the presence of edematous tissue, haemorrhage, proliferation of leucocytes, tubular necrosis, interstitial edema and atrophy of interrenal tissue. Fish exposed to lower doses (2.565 mg/l, 6.413 mg/l) showed glomerular swelling, edematous interrenal tissue, tubular necrosis and leucocyte aggregation. At higher concentrations (0.106 µg/l, 0.212 µg/l) lamda-cyhalothrin induced interstitial atrophy, collapse of glomeruli, tubular necrosis and proliferation of leucocytes whereas thiamethoxam at higher concentrations (12.826 mg/l, 25.651 mg/l) caused haemorrhage, tubular necrosis, glomerular swelling, proliferation of leucocytes and atrophy of interrenal tissue.
Earlier findings of many authors were in support of the histological observations of the present work. Dhanapakiam and Premalatha, (1994) found deformed glomeruli, necrosis, degenerated renal components in malathion and sevin treated *C. carpio*. Wannee *et al.* (2002) reported dilation of Bowman’s space in Nile tilapia exposed to herbicide Roundup. Cengiz (2006) observed degeneration in the epithelial cells of renal tubules, dilation of glomerular capillaries, degeneration of glomeruli, intra cytoplasmic vacuoles in epithelial cells of renal tubules with hypertrophied cells. Degeneration of glomerulus and vacuolization of the epithelial cells of uriniferous tubules were the endosulfan induced changes in the kidney of *Labeo rohita* (Indirabai *et al.*, 2010).

Kidney of the fish received larger portion of the post branchial blood for the removal of nitrogenous wastes and resulted in renal lesions. On the basis of the histological observations, it may be suggested that the abnormalities produced by the pesticides in kidney might be the reflection of osmotic imbalance as suggested by Khillare and Wagh (1985). Several xenobiotics could initiate the formation of specific enzymes that cause changes in metabolism, leading to cellular intoxication and death, at a cellular level called as necrosis. According to Dubale and Shah, (1984) the consequent necrosis of cell and vacuoles formation were due to precipitation and reduction of protoplasmic materials. Increase in leucocytes may have been an inflammatory response to pesticides. Leucocytes engulf injured and non- functional cells.

Similar to gill, intestine also served as a port of entry for the pesticides and different regions of the gastro intestinal tract were affected by the toxic chemicals. Haemorrhage, necrosis, edema, vacuolization of mucous epithelium and accumulation of macrophages were the significant changes found in the intestine of *C. carpio*
treated with lower doses of lambda-cyhalothrin and thiamethoxam. Exposure to higher concentrations of lambda-cyhalothrin resulted in necrosis, sloughing of mucosal layer, edematous zones, vacuolization, accumulation of macrophages, atrophy and desquamation of intestinal epithelium. Thiamethoxam at higher levels induced sloughing of mucosal layer, degenerative changes in epithelial lining, atrophy and infiltration of macrophages in the sub mucosal layer of the intestine. Since intestinal villi participate in absorptive function, extensive damage caused to the intestine definitely would affect all the metabolic activities in fish.

The pathological alterations in the intestine of the studied fish were in agreement with those changes observed in several fish species exposed to different pesticides. Recent report by Leena, (2014) in *C. carpio* treated with fenthion showed vacuolation, lesion in epithelial cells of villi, ruptured tips of villi and cellular exudates. Disjointment of layers and shortened villi were reported in dimethoate treated *Clarias batrachus* (Sajda *et al*., 2014). Deltamethrin induced necrosis, infiltration of lymphocytes and eosinophils in the intestine of *Gambusia affinis* was documented by Cengiz and Unlu, (2006). Epithelial degeneration and inflammatory cells infiltration in the sub mucosal layer as well as sub mucosal edema were seen in the intestine of tilapia exposed to carbofuran (Soufy *et al*., 2007). Eosinophilic granular infiltration in the sub mucosa, epithelial degeneration and sub mucosal edema were observed in edifenphos treated *Oreochromis niloticus* (Gaafar *et al*., 2010).

Significant changes observed in the muscle of lambda-cyhalothrin (1/50th and 1/20th of LC50) treated fish included inflammation, myofibrillar necrosis, thickening and separation of bundles of muscle fibres and variation in fibre size. Higher concentrations (1/10th and 1/5th of LC50) of lamda-cyhalothrin resulted in
degenerative changes and infiltration by macrophages. Intramuscular edema, necrosis, thickening and separation of muscle bundles were observed in thiamethoxam treated fish at lowest dose (1/50\textsuperscript{th} of LC\textsubscript{50}) whereas muscular atrophy and macrophage infiltration were the prominent changes found at other doses.

In support of the results of the present study, the following reports of some authors were given. Intramuscular edema, dystrophic changes with thickening and separation of muscle bundles were documented in carp exposed to HCH compounds (Das and Mukherjee, 2000.b.). Breakdown of muscle fibres and vacuolation were observed in alpha- cypermethrin treated \textit{Catla catla} (Muthuviveganandavel \textit{et al.}, 2013). Dimethoate induced changes in \textit{Clarias batrachus} were edema, mild lymphocyte infiltration, vacuolar degeneration, edema between muscle bundles and splitting of muscle fibres (Sajda \textit{et al.}, 2014). Dimethoate caused separation and degeneration of muscles, atrophy of muscle bundles and focal area necrosis at lower dose and vacuolar degeneration and splitting of muscle fibre at higher dose in \textit{Oreochromis mossambicus} (Pragna \textit{et al.}, 2010).

In the present work, lambda- cyhalothrin induced edema, vacuolation and macrophage infiltration in the brain of \textit{C. carpio} exposed to lower concentrations (0.021 µg/l and 0.053 µg/l). Addition to these changes, neuronal degeneration and reactive gliosis were found at exposure to higher concentrations (0.106µg/l and 0.212 µg/l). Thiamethoxam at lower doses (2.565 mg/l and 6.413 mg/l) brought edematous zones and vacuolation in brain tissue. Macrophage infiltration and degenerative changes were found at higher concentrations (12.826 mg/l and 25.651 mg/l). The result indicated that exposure to sublethal concentrations of lambda- cyhalothrin and thiamethoxam to \textit{C. carpio} might have produced a direct effect on the brain tissue thereby affecting its metabolism. Glycolysis leading to
microsomal and mitochondrial dysfunctions might have caused the vacuolation and glial cell reaction proved the toxic nature of the chemical (Das and Mukherjee, 2000b.). These pesticides are neurotoxins which inhibited acetylcholine in neuronal synapses and affected the functioning of motor coordination of the fish body. This contributed to many of the behavioural changes of the treated fish.

Similar alterations were recorded in C. carpio exposed to HCH by Das and Mukherjee, (2000). Lower concentration caused mild vacuolar changes and higher concentrations resulted in severe necrosis of neuronal cells of cerebrum as well as accumulation of glial cells. Houjuan et al. (2012) reported damage in the brain cells of common carp treated with atrazin and chlorpyriphos. Neuronal degeneration and necrotic areas in brain tissue of Oreochromis niloticus treated with edifenphos was reported by Gaafar et al. (2010). Channa punctatus exposed to chlorpyrifos showed degeneration of neuronal cells, spongosis, necrosis, appearance of clear areas around mononuclear cells and vacuolation (Mishra and Devi, 2014) and to endosulfan showed mild necrosis of the cerebrum and gliosis in cerebrum (Sarma et al., 2010). Chlorpyrifos induced changes in Catla catla were necrosis and vacuolation (Namdeo et al., 2013) and necrosis, atrophy and vacuolation (Tilak et al., 2005). Pugazhvendan et al. (2009) recorded severe damage in brain cells with neural bundles in malathion exposed Ophiocephalus punctatus.

Gonads of fish exposed to pesticides in this study revealed many histopathological alterations. Testis of C. carpio exposed to lambda- cyhalothrin showed vacuolation, condensation of tubular cells, increased interlobular areas, infiltration of leucocytes at lower concentrations (1/20th and 1/50th of LC50) and disintegrated basement membrane, regressed and disintegrated tubules at higher concentrations (1/5th and 1/10th of LC50). Significant changes in thiamethoxam treated
fish were vacuolation, necrosis, presence of foam cells and increased interlobular areas (1/20\textsuperscript{th} and 1/50\textsuperscript{th} of LC\textsubscript{50}) and condensation of tubular cells, disintegrated basement membrane, disintegrated tubules, regressed and diffused tubules (1/5\textsuperscript{th} and 1/10\textsuperscript{th} of LC\textsubscript{50}). Similar changes were observed in the testis of various pesticide treated fishes by many investigators. Sahai, (1992) reported testicular degeneration, atrophy, damaged connective tissue, degenerated interstitial cells in BHC treatment, distorted tubular structure, vacuolation of seminiferous tubules in endosulfan treatment and ruptured tubules displaying necrosis in malathion treatment in \textit{Puntius ticto}. Necrosis of interstitial cells in \textit{Glossogobius giuris} exposed to fenthion (Zutshi, 2005); necrosis and intertubular vacuoles in \textit{Oreochromis mossambicus} exposed to dimethoate (Desai \textit{et al}., 2011); foam cells in lobular lumen and infiltration by macrophages in \textit{Cichlasoma dimerus} exposed to endosulfan (Da Cuna \textit{et al}., 2013) and damage in intertubular connective tissue, degeneration of Leydig cells and disorganization of testis in \textit{Labeo rohita} exposed to endosulfan (Archana and Shashikant., 2014) were some of the results documented regarding pesticide toxicity in several fishes. In the present study presence of foam cells in the testis showed the sign of degenerative processes and macrophages ingest the fragments of tissue injury which resulted in cell debris and plasma membrane fragments and acquired foam cells characteristics (Da Cuna \textit{et al}., 2013). Initiation of necrosis caused the appearance of intertubular vacuoles. As reported by many authors in a variety of fishes, pesticides damaged the seminiferous tubules or destroyed the intercellular Leydig cells or both which resulted in the partial or total arrest of spermatogenesis (Desai \textit{et al}., 2011).

Significant changes in the ovary of \textit{Cyprinus carpio} treated with lambda-cyhalothrin at lower concentrations (0.021 µg/l and 0.053 µg/l) were necrosis, nuclear retraction, atresia, vacuolation in cytoplasm of the oocytes and degenerative
changes in pre vitellogenic and vitellogenic oocytes. Higher concentrations (0.106 µg/l and 0.212 µg/l) induced changes like increased atresia, empty follicles and expelled nuclei, decrease in size of oocytes, degeneration of oocytes, infiltration of macrophages and large interfollicular space. Thiamethoxam (2.565 mg/l) caused alterations such as increased interfollicular space, damaged ovigerous lamellae, empty follicles with expelled nuclei and nuclear retraction. Presence of free floating follicular lining, nuclear retraction, atresia, damaged ovigerous lamellae and increased interfollicular space in ovary were induced by 6.413 mg/l thiamethoxam. Significant changes caused by higher concentrations (12.826 mg/l and 25.651 mg/l) were reduction in size of oocytes, disruption and vacuolation in cytoplasm, atresia, empty follicles and large interfollicular space.

The findings of the present work are in concurrence with the histopathological alterations of ovary induced by pesticides in fish obtained by many authors. Necrosis, empty follicles, atresia, macrophage infiltration, frayed and broken ovigerous lamellae, decrease in size of oocytes in endosulfan treated *Lepomis macrochirus* (Dutta and Dalal, 2008); disruption of follicular epithelial cells and vacuolation in dimethoate treated *Oreochromis mossambicus* (Desai et al., 2011); vacuolation of oocytes and atresia in basathrin treated *Heteropneustes fossilis* (Giri et al., 2000); necrosis, atresia, clumping of cytoplasm, degeneration of pre vitellogenic and vitellogenic oocytes in endosulfan treated *Labeo rohita* (Archana and Shashikant, 2014); reduction in size of mature oocytes, disruption and vacuolation in cytoplasm and necrosis in malathion treated *Channa punctatus* (Magar and Bias, 2013) were documented. Rastogi and Kulshrestha, (1990) reported carbofuran, endosulfan and methyl parathion induced necrosis, damage to yolk vesicles of maturing oocytes in minnow, *Rasbora daniconius*. Ganeshwade, (2013) observed clumping of cytoplasm,
necrosis, atresia, increased interfollicular space and absence of matured oocytes in dimethoate treated *Puntius ticto*. Deka and Mahanta (2012) reported clumping, cytoplasmic degeneration, increased atretic oocytes, partial destruction of ovigerous lamellae in malathion treated *Heteropneustes fossilis*.

Pesticides in their sublethal concentrations affected different tissues and brought about architectural changes which made the organism less suitable for survival. The changes found could affect fish health and the fish became more sensitive to pesticides. Therefore it should be emphasized that pesticides even at low dosage could penetrate deep into different body tissues and destructed the cellular structure.