CHAPTER - VI

HISTOPATHOLOGICAL STUDIES
Histopathological Changes

VI.1 Introduction

Due to urbanization, industrial activities, freshwater sources are contaminated with different types of chemicals that affect the inhabiting biota. The complexity of the contaminants may induce a variety of biological responses (Tilli et al., 2010). Aquatic ecosystem is the final sink for the many chemicals used in industry and agriculture has a global problem, the continuous release of these chemicals impair water quality and become unsuitable for aquatic organisms due to their persistence, bioaccumulation, toxicity and biomagnification in food chain and ecological balance (Palaniappan and Karthikeyan, 2009; Subramani et al., 2011). Pesticide residues often reach the aquatic ecosystem and can be transferred to phytoplankton to fish and ultimately to humans (Sancho et al., 2010).

The toxicity of any environmental contaminant is either acute or chronic (David Briggs, 2003). The chronic studies include both activities of the organisms; physiological studies alone do not satisfy the complete understanding of pathological conditions of tissue under toxic stress. Hence, it is useful to have consequence of the concentration of the toxicant and is time dependent. The damage of particular tissue depends on the toxic potentiality of a particular contaminant accumulated in the tissue (Ashraf M. Abdel-Moneim et al., 2012). Disturbance of the homeostasis of an organism leads to compensatory, adaptive, and finally pathological processes, which are mostly energy-demanding. Therefore the metabolic rate of an organism may increase under toxic stress (Callow and Sibly, 1990; Dutra et al., 2009).
Over the last two decades, biomarkers have been widely used to assess the exposure and effects of environmental contamination. Exposure of an organism to a toxicant could involve several bio-chemical, physiological responses and pathological changes, which may lead to toxicity. These biochemical processes present the most sensitive and relatively early events of pesticide damage (Begum, 2004).

Aquatic organisms are sensitive to pesticide chemicals, and toxic concentrations may raise not only from excessive spillage of agricultural practices but also from several other sources. Apart from causing death either directly or due to starvation by destruction of food organisms, many pesticides has been show to effect growth rate, reproduction and behavioral responses with the evidence of tissue damage. Most of the histopathological changes can be interpreted as non-specific response to stress and a wide spectrum of pollutants, including pesticides, heavy metals, and organic contaminants exposed to fish (Pereira et al., 2013).

Fish contamination is a reliable indicator of bioaccumulation of persistent toxic substances in the environment, and has been used to estimate contaminant exposure risk to higher trophic levels, including humans and piscivorous wildlife (Ackerman, 2008; Stahal et al., 2009). Fish are important organism for the transfer of contaminants to human populations and may indicate the potential exposure to pollutants (Aharf M.Abdel-Moneim et al., 2012). In fact, they are suitable bioindicators since often they are key species in aquatic ecosystem and several species respond rapidly to abiotic changes, including pollution (Karen, 2010).

Different biomonitoring programs have used the histological changes observed in fish tissues as biomarkers of aquatic ecosystems (Pinto et al., 2010). Histopathological
studies allowed the identification of several changes induced by environmental pollutants (Monterio et al., 2008). Histopathological studies have proved to be a sensitive tool to detect direct effect of toxicants within in target organs of fish (Costa et al., 2011; Yasser and Naser, 2011). The toxic effects of pesticides in various forms ranging from a single cell, whole organism or whole population (Lazhar et al., 2012). Aquatic vertebrates are susceptible to non-target effects, because of their relatively restricted mobility and also due to reduced pesticide dispersion leading to lengthy periods of exposure (Smith and Straltar, 1986).

Histopathological investigations on different tissues of fish are valuable tools for toxicology studies and monitoring water pollutions. In histopathology, can provide information about the health status and functionality of different organs. Tissue injuries and damages in organs can result in the reduced survival, growth and fitness, the low reproductive success or increase of susceptibility to pathological agents. Frequency and intensity of tissue lesions depend on the concentrations of pesticides and the length of the period fish are exposed to toxicants. Nevertheless, many insecticides cause specific or non-specific histopathological damage (Fanta et al., 2003).

Histopathological lesions in the liver tissue of freshwater fish Cirrhinus mrigala investigated by (Velmurugan et al., 2009) were observed after 10 and 30 days exposure to sub lethal concentrations of dichlorvos and diazinon insecticides, respectively. Other researchers reported the same histopathological alterations in different tissues of fish treated with diazinon (Banaee et al., 2011), deltamethrin (Cengiz, 2006; Cengiz and Unlu, 2006), fenitrothion (Benli and Özkul, 2010).
Though a number of pathological changes had been reported in fish exposed to different pesticides (Inabamani et al., 1998; Jaun B. Ortiz et al., 2003; Capkin et al., 2010; Velcheva et al., 2010; Metwally Montaser et al., 2010; Anita Susan et al., 2011; Banaee et al., 2012; Pereira et al., 2013; Maharajan et al., 2013; Gonca Alak et al., 2013), hence no characteristic trends of pathological changes for any class of pesticides were distinct.

Histology and histopathological changes provide to be a sensitive tool to detect effect of chemical compounds, irritants within the target organs of the fish and other aquatic invertebrates (Costa et al., 2011; Yasser and Naser, 2011). Hence, in the present study, an attempt has been made to observe possible histopathological changes in certain vital tissues like gill, liver, kidney and brain of the freshwater fish *L. rohita* exposed to lethal (96 hr LC$_{50}$) and sublethal concentration (1/10$^{th}$ of 96 hr LC$_{50}$) of profenofos and carbosulfan commercial grade formulations for 24 hr and 8 days.

**VI.2. Materials and Methods**

The common edible fish, *Labeo rohita* is obtained from the local fish farm at Nandivelugu, Guntur District of Andhra Pradesh, India. The length of the fish 6±7 cm, average body weight 6.5± 7.5g. The fish *L. rohita* were acclimatized to the laboratory conditions at 28±2°C for 15 days. The freshwater fish, *L. rohita* were exposed to sublethal concentrations (10 µg l$^{-1}$; 0.12 mg l$^{-1}$) and lethal concentrations (100 µg l$^{-1}$; 1.2 mg l$^{-1}$) of organophosphorus pesticide profenofos and carbamate pesticide carbosulfan for 24 hr and 8 days. At the end of the exposure period, fish were randomly selected for histopathological examination.

Gill, liver, kidney and brain tissues were isolated from control and both toxicants treated fish. Physiological saline solution (0.75% NaCl) was used to rinse and clean the
tissue. They were fixed in aqueous Bouins solution for 48 hr processed through graded series of alcohols cleared in xylene and embedded in paraffin wax. Gills alone were processed by double embedding technique. Sections were cut of 6 μ (microns) thickness; stained with Ehrlich’s haematoxylin and Eosin (dissolved in 70% alcohol) (Humason, 1972) and were mounted in Canada balsam. Histopathological lesions were examined and photographed with the help of Olympus computer attached microscope under 400 x lenses.

VI.3. Observations and Discussion

VI.3.1. General histology of fish gill

Teleosts have five pairs of gill arches. The primary gill filamentous in each arch form two rows and joined at the base by a gill septum. The last pair of gill arches generally transforms into the pharyngeal bone and does not play a role in respiration. The primary gill lamella (PGL) is flat leaf like structures, with a central rod like supporting axis (CA) with a row of secondary gill lamella (SGL) on each side of it (Plate VI.3.1 and Fig. A).

They are situated laterally on either side of interbrachial septum. The secondary gill lamella is also known as respiratory lamella. The surface is covered with simple squamous epithelial cells separated by mucous cells erythrocytes and is highly vascularised. Blood vessels can been seen extended into each secondary gill lamella. The blood cell has a single nucleus, which is flattened in appearance. The region between two adjacent respiratory lamella is termed as interlamellar region.
VI.3.1.1. Pathology of Gill tissue under profenofos and carbosulfan toxicity at sublethal and lethal concentrations

Profenofos and carbosulfan caused marked pathological changes in the gills of exposed fish. They include atrophy, hydropsy, vascular degenerations bulging of tips of primary gill filaments and vascular degenerations, curling of secondary gill filaments was also observed, the pillar cell nucleus showed necrosis, severe necrotic changes in the epithelial cells of secondary gill lamellae and formation of vacuoles in the secondary gill epithelium. There is a tendency of fusion of disorganized secondary gill filaments (Plate VI.3.1 and Fig. B-F).

Marutirao, (2012) reported slight hyperplasia of gill epithelium in dimethoate exposed to Puntius ticto (Ham.), a commonly used organophosphate. Inflammatory alterations of lamellar epithelium and hyperplasia were reported in the gill of freshwater Cyprinus carpio communis (L) exposure to sublethal concentration of lead and cadmium (Paitnaik et al., 2011). Edema with lifting of lamellar epithelium and hyperplasia of lamellar epithelium were observed in the gills of yellow perch (Perca flavescens) and gold fish (Carassius auratus) polluted water containing residues of oil sands (Nero et al., 2006). Similar findings were noted in the gills of White seabass Lates calcarifer on exposure to acute and chronic cadmium by Thophon et al., (2003). Monteiro et al., (2008) reported swellings of inflammation in almost all the respiratory lamellae of gills of Oreochromis niloticus on exposure to copper.
Legend for Figures

Plate VI.3.1

Fig. A. Control: Normal Gill lamella of *L. rohita* after 24 hr; Bouin, Scale bars = 50µm, HEx 400

- PGL: Primary gill lamella
- GA: Gill arch
- SGL: Secondary gill lamella
- GF: Gill filaments
- PC: Pillar cells

Fig. B & F. Profenofos Sublethal: Normal Gill lamella of *L. rohita* exposed to sublethal concentration of profenofos for 24 hr and 8 days; Bouin, Scale bars = 50µm, HEx 400

- CU: Secondary lamellar curling
- HP: Hyperplasia
- MCD: Marginal Cell Dilation
- BC: Blood Congestion
- DGSPL: Degenerated primary lamella
- DGSL: Degenerated Secondary lamella
- N: Necrosis
- F: Fusion

Fig. D & G. Carbosulfan Sublethal: Normal Gill lamella of *L. rohita* exposed to sublethal concentration of carbosulfan for 24 hr and 8 days; Bouin, Scale bars = 50µm, HEx 400

- DGSL: Degenerated Primary gill lamella
- EH: Epithelial Hyperplasia
- DCD: Degenerated Chondrocyte
- BC: Blood Congestion
- DGSL: Degenerated Secondary lamella
- N: Necrosis

Fig. C & E. Profenofos and Carbosulfan Lethal: Normal Gill lamella of *L. rohita* exposed to lethal concentration of profenofos and carbosulfan for 24 hr; Bouin, Scale bars = 50µm, HEx 400

- ED: Epithelial desquamation
- LT: Lamellar telangiectesis
- CU: Secondary Lamellar curling
Plate VI.3.1

Fig. A. Control Gill

Fig. B. Profenofos sublethal 24 hr

Fig. C. Profenofos lethal 24 hr

Fig. D. Carbosulfan sublethal 24 hr

Fig. E. Carbosulfan lethal 24 hr

Fig. F. Profenofos sublethal 8 days

Fig. G. Carbosulfan sublethal 8 days
Hyperplasia, desquamation, and necrosis of epithelial lifting edema and collapsed secondary lamellae were observed in the freshwater fish *Cirrhinus mrigala* exposed to dichlorvos (Velumurugan *et al.*, 2009). Santos *et al.*, (2012) observed toxicity of Formalin cause of pathological damage in the gill, gill dysfunction, osmoregulatory and respiratory imbalance in ornamental fish amazon blue spotted corydora (*Corydoras melanistius*). This is due to the gill epithelium is the primary contact surface, with the external environment, became a target of the environmental contaminants present in the polluted water. Athikesavan *et al.*, (2006), reported the infused secondary lamellae, hypertrophy of gill filament, hyperplasia of epithelial surface and severe erosion and aggregation of blood corpuscles in gill of freshwater fish, *Hypophthalmichthys molitrix* (Valenciennes) exposed to Nickel.

Anitha Kumari and Shree Ram Kumar, (1997) observed decreased carbohydrate activity in the secondary lamellae and also in the respiratory epithelium of the freshwater teleost *Channa punctatus* under exposure to the polluted water of Hussain Sagar and states that the degeneration of respiratory epithelium and damages of gill tissue causes a decrease in energy metabolism.

Hyperplasia of gill filaments, fusion of gill filaments due to separation of epithelium, necrosis of gill epithelium, degeneration of pillar cells, development of vacuoles in the epithelium are the pathological changes observed in profenofos and carbosulfan exposed fish. Similar changes were observed in rainbow trout exposed to sublethal concentrations of Monocrotophos (Vijayalakshmi, 1996) to fenvalerate in *Labeo rohita* (Tilak *et al.*, 2001) to cypermethrin in *Labeo rohita* (Veeraiah, 2001).
VI.3.2. General Histology of Liver

The surface of liver is covered with serous membrane and some connective tissue extends inwards into parenchyma. It is composed of parenchymal cells, hepatic cells and lattice fibers, which support the former. Hepatic cells are roundish polygonal, containing clear spherical nucleus. They are located among sinusoids forming cord like structures known as Hepatic cell cords. In fish these structures are generally obscure. Bile canaliculus is centrally located in each cord, fairly large quantities of lipid and Glycogen granules are also observed in the cytoplasm of fish hepatic cells.

Hepatic cells have many vital functions other than the secretion of bile. They play an important role in protein, lipid and carbohydrate metabolism. They serve as storage sites for some nutrients and detoxication is another important function.

VI.3.2.1. Pathology of Liver tissue under profenofos and carbosulfan toxicity at sublethal and lethal concentrations

Profenofos and carbosulfan has induced discrete pathological changes in the liver tissues. These changes include degenerations of cytoplasm in hepatocytes, atrophy, and necrosis, formation of vacuoles, rupture in blood vessels, necrosis and disappearance of hepatocyte wall and disposition of hepatic cords, formation of vacuoles, rupture in blood vessels, blood congestion, necrosis and disappearance of hepatocyte wall and disposition of hepatic cords, decrease in the size of nucleus as evident(plateVI.3.2 and Fig.B-F).
Legend for Figures
Plate VI.3.2

**Fig.A. Control:** Normal structure of Liver in *L. rohita* after 24 hr; Bouin, Scale bars = 50µm, HEx 400
N - Nucleus
HC - Hepatic Cell
GC - Granular Cytoplasm

**Fig.B&F. Sublethal profenofos:** Normal structure of Liver in *L. rohita*, exposed to sublethal concentration of profenofos for 24 hr and 8 days; Bouin, Scale bars = 50µm, HEx 400

EG - Eosinophilic granules
NH - Nuclear Hypertrophy
ISN - Irregular shaped nucleus
VF - Vacuole formation
PN - Pyknotic nuclei
SS - Increased sinusoidal Space
IO - Intracellular oedema
CN - Cell necrosis
N - Necrosis

**Fig.D&G. Sub lethal Carbosulfan:** Normal structure of Liver in *L. rohita*, exposed to sublethal concentration of carbosulfan for 24 hr and 8 days; Bouin, Scale bars = 50µm, HEx 400

PN - Pyknotic nuclei
SS - Increased sinusoidal space
VF - Vacuole Formation
EG - Eosinophilic Granules
ISN - Irregular Shaped Nucleus
IO - Intracellular oedema
N - Necrosis
VF - Vacuole formation

**Fig.C&E. Profenofos and Carbosulfan Lethal:** Normal Structure of Liver in *L. rohita* exposed to lethal concentration of profenofos and carbosulfan for 24 hr; Bouin, Scale bars = 50µm, HEx 400

ND - Nuclear Degeneration
CD - Cytoplasmic Degeneration
IO - Intracellular oedema
MA - Melanomacrophages Aggregate
CN - Cell necrosis
VF - Vacuole formation
Liver, is the primary organ for metabolism, detoxification of toxic substances, removal of harmful substances, individuals containing sub lethal concentrations of pesticides such as profenofos and carbosulfan, showed necrosis swelling of heptocytes, congestion, vacuolar degeneration, karyolysis, dilation of sinusoids and pycnotic nucleus due to these reasons the hepatic cells severely damaged.

Tulasi and Jayantha Rao, (2012) observed cytoplasm with nuclear degeneration; cellular degeneration and damaged hepatocytes in fish *Cyprinus carpio* exposed to chromium. Velumurugan *et al.*, (2009) reported that necrosis of hepatocytes with enlarged sinusoid in freshwater fish *Cirrhinus mrigala* exposed to dichlorvos. Ray and Bhattacharya, (1985) reported that nuclear degeneration, hepatic cord disarray and precipitation of cytoplasm in *Anabas testudineus* teleost exposed to acute and chronic levels of cythion. John *et al.*, (1993) reported that extensive vacuolation, indistinct cell boundaries, degenerative necrosis in fish *Cyprinus carpio* exposed to endosulfan.

El-Sayed M.A.El-Damaty (2012) reported that focal necrosis with inflammatory infiltration, vacuoles in the hepatocytes and hepatic sinusoids congestion in albino rats treated with dimethoate. Formation of vacuoles, cytoplasm in hepatocytes, atrophy, blood streaks among hepatocytes, intercellular empty space and disintegration of lattice fibers were observed in fish *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* exposed to fenvalerate by Anita Susan *et al.*, (2012). Velcheva *et al.*, (2010) reported that bleak (*Alburnus alburnus* L.), rudd (*Scardinus Erythrophthalmus* L.) and perch (*Perca fluviatilis* L.) fish inhabiting waters with different heavy metals pollution, showed the granular degeneration and necrotic changes in liver. Juan B. Ortz *et al.*, (2003) reported that in *Cyprinus carpio* and *Barbus* sp exposure to lindane caused hepatocellular necrosis with
parenchymal vacuolization; hypertrophy of hepatocytes was damaged in liver. Velumurugan et al., (2009) reported significant alterations in the hepatocytes, pyknotic nuclei and necrosis in the liver of *Clarias gariepinus* fish exposed to cypermethrin. Amminikutty and Rege (1977), reported that rapid degeneration and vacuolation of hepatocytes in liver tissue of fish Widow tetra (*Gymnocorymbus ternetzi*) (Boulenger) exposed to thiodon and Agallol. A few other reports are available which deal with the other pesticides effect on histology. Cruz (1989) reported that formalin treatment caused cloudy swelling haemorrhage, deposition of pigments and necrosis in liver of milk fish, Chanos fingerlings. Amalia Mitsoura et al., (2013) reported moderate focal necrosis, granular glycogen, nuclei pyknosis, loss of the architecture structure, onion-like cells in fish *Cyprinus carpio* due to presence of microcystins. Mohammad M.N. Authman (2011), observed the cytoplasmic degeneration in hepatocytes, formation of vacuoles, rupture in blood vessels and appearance of blood cells among hepatocytes, formation of vacuoles, pyknotic nuclei in the liver of *Oreochromis niloticus* exposed to aluminum. Similar changes were observed in rainbow trout under carbosulfan toxicity.

Profenofos and carbosulfan has induced discrete pathological changes in the liver of the pesticide treated fish. The pathological changes noticed in the liver might effect the physiological activity of the fish such as metabolic activities and enzymatic systems. This reduces the functional ability of liver, which indirectly effects all metabolic activities of the organism.

In this present study, chief histopathological changes in the liver were cell necrosis, formation of vacuoles, disposition of hepatic cords, sinusoidal space, disposition of
hepatic cords decrease in size of nucleus, intracellular edema, lipid infiltration, disappearance hepatocyte wall and pycnotic nucleus was evident (Plate VI. 3.2).

VI.3.3. General Histology of fish Kidney

Teleostean kidney consists of head and body kidneys. Head kidney is composed of a variety of cells, the anterior portion of the kidney and consists of lymphoid tissue and hematopoietic tissue (Mela et al., 2007). Body kidney is composed of many nephrons and interstitial lymphoid tissue. The interstitial tissue is the major haematopoietic tissue in the body. The main functional unit of kidney is nephron. Each nephron consists of two parts, the glomerulus and the urinary tubules (Plate VI.3.3). The glomerulus capsule consists of an inner and outer layer of single flattened epithelia. Renal tubules consist of a single layer of epithelial cells. Mesangium fills the space between the loops of glomerular capillaries. Renal tubules are thin and short in the neck segment. The proximal convoluted segment is divided into two parts - Segment II and I. The renal tubules are composed of cuboidal epithelial cells with densely arranged microvilli in the tubular lumen. In segment II renal tubules are composed of cuboidal epithelial cells.

Cilia and microvilli are found in the tubular lumen. In the distal convoluted segment epithelial cells have no microvilli. The cells of this segment are stained with eosin more faintly than those of proximal convoluted segment. Thus, it is easy to distinguish between proximal and distal convoluted segments under light microscopy (Oguri, 1982).
VI.3.3.1. Pathology of Kidney tissue under profenofos and carbosulfan of sublethal and lethal concentrations

Profenofos and carbosulfan toxicity caused marked pathological changes in the kidney of exposed fish, which include severe necrosis, degenerative changes in haemopoietic tissue, cloudy swelling in renal tubules, cellular hypertrophy and granular cytoplasm were evident. The epithelial cells of the distal convoluted tubules decreased in size. The interstitial renal tissue was less affected. Renal interstitial tissue showed formation of vacuoles and cellular contours were not clearly distinguished (plate VI.3.3 and Fig.B-F).

Banaee et al., (2013) reported that disorientation in glomerular structure, cloudy swelling, dilation in the inter space of urinary tubular, necrosis in the hematopoietic tissue, appearance of vacuoles in the cytoplasm epithelial cells of renal tubules and narrowing of the tubular lumen due to diazinon toxicity in fish rainbow trout (Oncorhynchus mykiss).

Necrosis in glomerular cells and haemopoietic tissues and degeneration of renal tubules, separation of tubular epithelium and contraction of glomerulus in fish kidney exposed three commercial grade pesticides such as carbosulfan, dithiocarbamate, fungicide propineb reported by Capkin et al., (2010).
Legend for Figures

Plate VI.3.3

Fig. A. Control: Normal structure of Kidney in L. rohita after 24 hr; Bouin, Scale bars= 50µm, HEx 400

RC - Renal Corpuscle (showing glomerulus & bowmen’s space)
PT - Proximal Tubule
DT - Distal convoluted tubule

Fig. B & F. Sublethal: Structure of Kidney in L. rohita, exposed to sublethal concentration of profenofos for 24 hr and 8 days; Bouin, Scale bars= 50µm, HEx 400

IC - Interstitial cells
GC - Glomerular congestion
TAL - Tubular Architectural loss
NHT - Necrotic haemopoietic tissue
DGPT - Degenerated primary tubule
CG - Contraction of the glomerulus
VF - Vacuole formation

Fig. D & G. Sublethal: Structure of Kidney in L. rohita, exposed to sublethal concentration of carbosulfan for 24 hr and 8 days; Scale bars= 50µm, Bouin, HEx 400

RC - Renal corpuscle (showing glomerular expansion & absence of Bowman’s space)
RTD - Renal tubular degeneration
DGPT - Degenerated primary tubule
VF - Vacuole Formation
GC - Glomerular congestion
HDD - Hyaline droplet degeneration
TCN - Tubular cell necrosis
STE - Separation of tubular epithelium

Fig. C & E. Profenofos and carbosulfan lethal: Structure of Kidney in L. rohita exposed to lethal concentration of profenofos and carbosulfan for 24 hr; Bouin, Scale bars= 50µm, HEx 400

VC - Vacuolated cytoplasm
OT - Occlusion of Tubular lumen
CSD - Cloudy Swelling Degeneration
HDD - Hyaline Droplets Degeneration
VF - Vacuole Formation
RTD - Renal tubular degeneration
NHT - Necrotic haemopoietic tissue
CG - Contraction of glomerulus
Plate VI.3.3

Fig. A. Control Kidney

Fig. B. Profenofos sublethal 24 hr

Fig. C. Profenofos lethal 24 hr

Fig. D. Carbosulfan sublethal 24 hr

Fig. E. Carbosulfan lethal 24 hr

Fig. F. Profenofos sublethal 8 days

Fig. G. Carbosulfan sublethal 8 days
Altinok et al., (2006) observed tubular necrosis and renal tubules were filled with
eosinophilic material in kidney of fish rainbow trout exposed to methiocarb. Boran et al.,
(2012) reported that reduced glomerular filtration rate, glomerular lesions, degeneration
of cellular boundaries and clumping of glomeruli at some places in the kidney of rainbow
tROUT (Oncorhynchus mykiss) exposed to fungicide captan.

Cloudy swelling of renal tubule marked Denisoni exposed to phosphamidon
(Rashatwar and Yas, 1984). In monocrotophos treated mice thickening of glomerular
basement membrane, tubular degeneration and compensatory dilation and the fecal
collection of chronic inflammatory cells in the interstitial tissue were reported by Malaya

Capkin et al., (2006) reported that rainbow trout exposed to endosulfan and
remarked that melanomacrophage centers were scattered throughout the trunk kidney.
Sayed et al., (2012) observed that blood congestion in between tubules and hemorrhage
in the interstitium albino rats treated with dimethoate, carbendazim or carbofuran
pesticides.

Degenerative changes in epithelial cells of proximal tubules and haemopoietic
tissues, severe necrosis in the proximal tubules leading to the formation of vacuoles,
degenerative changes in epithelial cells of collecting tubules of Tilapia mossambica
exposed to fenvalerate has been reported by Radhaiah (1989).

Furhan Iqbal et al., (2001) reported that cellular shrinkage and nuclear pycnosis,
disintegration of parietal layer of glomerulus, disorganization of proximal tubule. The
renal tubules of nuclei were hyper chromatic and in several cells were displaced to an
apical position in fish Cyprinus carpio exposed to nitrate.
Diethyl phthalate induced changes in fish *Clarias gariepinus* had necrosis of epithelial cells of renal tubule, pyknotic nuclei in haemopoietic tissues and degeneration of glomerulus reported by Ikele *et al.*, (2011).

The present investigations are in agreement with the reports of Bhattacharya *et al.*, (2008); Hajrudin Beširović *et al.*, (2010); who observed renal damage, rupture in the glomeruli and reduced renal tubules and reduced lumen in Brown Trout (*Salmo trutta m. fario*) and Rosy Barb (*Puntius conchonius*) and amphioxus (*Branchiostoma belcheri*). Epithelial swelling, swelling of mitochondria in the renal tubules and necrosis was also reported in animals administered with methothrin and pyrethrins respectively Foeng *et al.*, (1982). Such sort of pathological conditions causing dysfunction of kidney tissue have been reported under pesticide toxicity by Capkin *et al.*, (2012). Vardhani and Gouri (2002) reported that the histopathological changes in liver and kidney of *Labeo rohita* intramuscularly injected with Bovine serum albumin (BSA) pathogeny was evident by the destruction of hepatic cords and tubular cells of the kidney in experimental animals.

These results conform to the fenvalerate and heavy metals effects on kidney tissue of Indian major carps, *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* that have been reported by Anita Susan, (2012); Vinodhini and Narayanan, (2009).

**VI.3.4. General Histology of fish Brain**

Fish brain consists of five major regions, such as telencephalon, diencephalon, mesencephalon, metencephalon and myelencephalon. The diencephalon contains the third ventricle and is composed of the epithalamus, thalamus and hypothalamus (Fig.VI.3.4.A). The epithalamus contains the ends of the nerve fibres from the telencephalon and also the habenula, which connects with the thalamus, hypothalamus.
and the olfactory areas of the telencephalon. The mesencephalon contains the centre of the visual sense, as well as the integration center between this sense and the other senses of locomotion. Mesencephalon occupies the interior portion of the dorsal wall of the fourth ventricle and is composed of a cortex and medulla. The mesencephalon is the integration center between the auditory sense and the sense of the lateral line. The main part of the myelencephalon, the medulla oblongata, is shaped like the spinal cord opened on its dorsal side.

VI.3.4.1. Pathology of Brain under profenofos and carbosulfan toxicity at sublethal and lethal concentrations

The histopathological changes in brain tissue include oedema, necrosis, atrophy, pyknosis, swelling of the axon, vacuolar degeneration, severe damage in brain cells and broken neural bundles, degenerated dorsal olfactory area, degenerated ventral olfactory area and blood streaks were observed (Plate VI.3.4 and Fig. B-F). Profenofos and carbosulfan pesticides are neurotoxic compounds, caused atrophy, loss of nissl substances, necrosis of neuronal cells, congestion of blood vessels and edema causes abnormalities in the circulation of blood. Similar findings were observed by Das and Mukherjee, (2000) in fish *Labeo rohita* exposed to hexachlorocyclohexane.

Bendary *et al.*, (2014) reported that neuronal degeneration of purkinje, neuronophagia, focal gliosis and encephalomalacia with demyleration of nerve fibers, hemorrhages, congestion of blood vessels and edema in male mice treated with profenofos and chloropyrifos. Pugazhvendan *et al.*, (2009) observed that disintegrated neural cells, severe necrosis, damaged brain cells and neural bundles in fish *Ophiocephalus punctatus* exposed to malathion. Ahmet Topal *et al.*, (2014) observed
that neuronal necrosis and edema in a purkinje layer of cerebellar regions of brain in fish brown trout treated with cadmium. Due to direct effect on brain tissue, increase in the production of reactive oxygen species (ROS) cause and protein, mitochondrial DNA, nuclear DNA and membrane damages. The composed lesion of ROS causes necrosis (Klein et al., 2003; Colak et al., 2011).
Legend for Figures

Plate. VI.3.4

**Fig. A. Control:** Normal structure of Brain in *L. rohita* after 24 hr; Bouin, Scale bars= 50µm, HEx 400

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<tr>
<td>DOA</td>
<td>Dorsal olfactory area</td>
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<td>VOA</td>
<td>Ventral olfactory area</td>
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<td>SA</td>
<td>Septal area</td>
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**Fig. B&F. Sublethal Profenofos:** Normal structure of Brain in *L. rohita* exposed to sublethal concentration of profenofos for 24hr and 8 days; Scale bars= 50µm, Bouin, HEx 400

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<tr>
<td>DDOA</td>
<td>Degenerated dorsal olfactory area</td>
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<td>DVOA</td>
<td>Degenerated ventral olfactory area</td>
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<tr>
<td>BS</td>
<td>Blood streaks</td>
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<tr>
<td>DSA</td>
<td>Degenerated septal area</td>
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**Fig. D&G. Sublethal Carbosulfan:** Normal structure of Brain in *L. rohita* exposed to sublethal concentration of carbosulfan for 24hr and 8 days; Scale bars= 50µm, Bouin, HEx 400

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<tr>
<td>DDOA</td>
<td>Degenerated dorsal olfactory area</td>
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<td>DVOA</td>
<td>Degenerated ventral olfactory area</td>
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<td>Blood streaks</td>
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<td>DSA</td>
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**Fig. C&E. Profenofos and Carbosulfan Lethal:** Normal structure of Brain in *L. rohita* exposed to lethal concentration of profenofos and carbosulfan for 24 hr; Bouin, Scale bars= 50µm, HEx 400

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>DDOA</td>
<td>Degenerated dorsal olfactory area</td>
</tr>
<tr>
<td>DVOA</td>
<td>Degenerated ventral olfactory area</td>
</tr>
<tr>
<td>BS</td>
<td>Blood streaks</td>
</tr>
<tr>
<td>DSA</td>
<td>Degenerated septal area</td>
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</table>
Ayoola and Ajani, (2008) reported that mononuclear infiltration, neuronal degeneration, discoloration, severe spongiosis in African catfish *Clarias gariepinus* exposed to cypermethrin. Similar findings were also observed by Sarma *et al*., (2009); Altinok and Capkin, (2007), in spotted murrel *Channa punctatus* and rainbow trout exposed to endosulfan. Swelling of pyramidal cells with binucleated nuclei, necrosis of neuronal cells of cerebrum indicates loss of nissal substances, vacuolar changes were observed in fish *Labeo rohita* and *Catla catla* exposed to Zinc, Chlorpyriphos and *Datura stramonium* reported by (Loganathan *et al*., 2006; Namdeo *et al*., 2013). Eosinophilic granule cells (EGC) in the meninges and cerebral cortex, degeneration of neuronal bodies, necrosis and apoptosis and glyosis observed by Wilson *et al*., (2008) in Cachama blanca (*Piaractus brachypomus*), alterations in brain tissue indicate the degenerative neuronal processes.

Tongo Isioma and Ezemonye Lawrence, 2013 reported that degeneration of dark stained Purkinje neurons, oedema, necrosis and vacuolar changes in the cerebrum in African toad *Bufo regularis* exposed to endosulfan. The present study was supported by the previous studies of Loganathan *et al*., (2006), reported zinc induced alterations in fish *Labeo rohita*, Patnaik *et al*., (2011) observed that neuronal cell degeneration, swelling of pyramidal cells, loss of nissl substances, vacuolization and dystrophic changes, due to glycolysis leading to microsomal and mitochondrial dysfunctions. Necrosis of neuronal cells in the cerebrum indicating loss of nissl substances evident in fish *Cyprinus carpio* exposed to lead and cadmium after 28 days. Similar findings were observed by Houjuan *et al*., (2012) in common carp exposed to atrazin and chlorpyrifos.
Profenofos and carbosulfan causes several injuries and damages to the brain, in the present study was more in lethal than in sublethal concentrations. Both toxicants inhibit the normal functioning of the body metabolism and behavioral changes, sensorial system like vision and smell, detection, attack and capture of prey, impair feeding, escape, and reproductive behavior all these functions are connected with various parts of the brain, all of them are altered due to AChE inhibition caused by these agrochemicals treated fish then leads to impaired neuronal dysfunction of central nervous system.

Thus, when fish are exposed to pesticides, they suffer irreparable architectural changes in various vital organs marking the fish less fit for better survival. These histopathological changes can alter various physiological activities of the fish such as release of various enzymes and the metabolic processes as evidenced from Chapter-IV.