Chapter – VI
Nowadays farmers are using a number of pesticides in the operation of agricultural and other commercial crops for more production and protection to eradicate or control the pests. The dependence of modern agriculture on agrochemicals like pesticides heavily is emerging as a threat to the ecological balance of aquatic ecosystems. Synthetic pesticides used for controlling pests in agriculture are one of the major causes of aquatic pollution. The residues of these pesticides mostly reach into aquatic ecosystems through surface run off and affect the health of non target organisms including fish. Agricultural runoff, effluents from pesticide manufacturing industries, or accidental spillage pose a serious threat to the aquatic environment, affecting aquatic lives including that of fish as well as other organisms (Dabrowski et al., 2002; Anasco et al., 2010).

Among synthetic pesticides organophosphates are widely used in agriculture and in health and hygiene programmes due to their high effectiveness as insecticides and less persistence in the environment. Organophosphate (OP) compounds are a diverse group of chemicals used in both domestic and industrial settings. They are favoured over organochlorines, which have long persistence and consequently easily bioaccumulate in food chain. The shift from organochlorines to organophosphates has resulted into increased occurrence of organophosphates into water bodies causing acute and chronic toxicity to fish fauna (Rao et al., 2005; Velmurugan et al., 2007; Singh et al., 2009). The health effects associated with organophosphate poisoning are a result of excess acetylcholine (ACh) present at different nerves and receptors in the body because acetycholinesterase is blocked.

Histology is the study of the microscopic anatomy of cells and tissues. It is the study of tissue sectioned as a thin slice, using a microtome. It has been successfully employed as a diagnostic tool in medical and veterinary science. Exposure of animals to contaminated water causes severe pathological changes at the tissues level. Interaction between pollutants and cellular components results in pathological changes in cellular and sub-cellular level as observed through histopathological analysis (Schwaiger et al., 1997).

The knowledge of the histology is useful to distinguish normal cells from abnormal or diseased ones, which helps in diagnosis of many diseases (Majumdar, 1980). The physiological investigations when coupled with cytoarchitectural studies, the toxicological studies seem to be complete so as to give a picture of the extent of pesticidal effect. The cytoarchitectural dynamics of a tissue is very essential for maintaining the tissue integrity and for effective physiological, biochemical and metabolic functions. The cellular and sub-cellular
constituents of tissue in terms of size, shape, number and position play an important role in the physiological and metabolic functions. Therefore, the histological structure of tissue in an animal has a profound influence on its function.

Histopathology refers to the microscopic examination of tissue in order to study the manifestations of disease. Toxicological histopathology gives useful data concerning the changes induced by chemicals at the tissue and cellular level. All the tissues and organs in the body of an animal may be potential targets for the toxic effects of any chemical compound. A histopathological assessment throws light on the nature of tissue alteration and the extent of damage. This in turn indicates the toxic nature of the compound. Therefore, histology gives useful insight into the tissue lesions to prove the external manifestations of the deleterious effects of toxic chemicals (Jayantha Rao, 1982). Although the qualitative data is used in most cases to study the histopathological effect, quantitative data shows a better understanding of the detailed mechanism of interaction between organism and pollutants at the molecular level (Jagoe, 1996).

Histopathology is the microscopic study of diseased tissue and is an important tool of anatomical pathology since accurate diagnosis of diseases usually requires histopathological examination of samples. Even though biochemical studies may give an idea of the pathological state of the animal, a clear picture of cytoarchitectural changes produced during the chemical intoxication can be traced by histopathological studies. Histopathological studies would help in assessing the extent of pollution in the ecosystem by pesticides and offer an exceptional opportunity to detect the effect of pollutants in various organs and organ system of an organism.

Pesticides possess high toxicity not only to target but also non-target organisms. These substances find their way into places far from application and lead to alterations in metabolic activities of living organisms by bio-accumulation. Pesticides find their way into places far from application and accumulate in significant concentrations in the tissues of animals. Pesticide residues in the tissues cause serious physiological alterations even at low levels (Dikshith et al., 1978; Mathur et al., 1981). Johnson (1973) and Verma et al., (1974) pointed out that a prolonged period of exposure to chemical compounds with very low concentration, results in the accumulation of more pesticide in the organs.

Pathological lesions due to lethal and sublethal effects of xenobiotic compounds are very important for delineating fish health status and for future ecological impact (Jiraungkoorskul et al., 2003). Specific lesions occurring in the organs of fish exposed to toxic
substances under laboratory conditions are helpful as biomarkers of exposure. As a result histopathological examination is increasingly being recognized as a valuable tool for assessing the impact of environmental pollutants like pesticides on fishes (Teh et al., 1997; Handy et al., 2002).

Susceptibility of a chemical injury varies greatly in the tissues and cells of the same animal and in different animal groups. However, the location of the major damage may be determined by the mode of action of the chemical. Some of the chemicals exert their effect locally at the portal of entry and some other toxic compounds do not cause damage at the portal entry but affect the organs systematically in which they are accumulated. The extent of severity of tissue damage is a function of the concentration and potentiality of toxic compound accumulated in the tissues as it is time dependent (Jayantha Rao, 1982).

Several reports are available on the cytoarchitectural damage in different organs of various animals exposed to pesticides (Radhaiah, 1988; Vani, 1991; Manoranjitham et al., 1993; Mohssen Morowati. 1997; Morowati, 1998; Shukla et al., 2001, Glynn, 2003; Garg et al., 2004; Sakr and Jamal Al lai, 2005; Madhavee latha, 2006; Sivaiah, 2006; Velmurugan et al., 2007; Rajendra Prasad, 2007; Sukanya, 2007; Velmurugan et al., 2007; Rajeswari, 2008; Babu Velmurugan et al., 2009a, 2009b; Tripathi et al., 2011).

Sakr and Jamal Al lai, (2005) studied the hazardous effect of the pyrethroid insecticide, fenvalerate on the histology and histochemistry of the liver of the catfish, Clarias gariepinus after exposure to 1/10 of LC50 for 5 and 10 days and reported that the histopathological changes induced in the liver were mainly represented by cytoplasmic vacuolization of the hepatocytes, blood vessel congestion, inflammatory leucocytic infiltration, necrosis and fatty infiltrations.

Velmurugan et al., (2007) studied the histopathological effects of monocrotophos on the gill, kidney and intestine tissues of the Cirrhinus mrigala by light microscopy and reported that the changes in the gills were characterized by epithelial hyperplasia, aneurism, epithelial necrosis, desquamation, epithelial lifting, oedema, lamellar fusion and curling of secondary lamellae. Pycnotic nuclei in tubular epithelium, hypertrophied epithelial cells of renal tubules, contraction of the glomerulus and expansion of space inside the Bowman’s capsule were observed in the kidney tissues of fish after exposure to monocrotophos. In the intestine tissues of fish exposed to monocrotophos, oedema, necrosis and atrophy of epithelial cells were observed.
Babu Velmurugan et al., (2009a) determined the histopathological effects of dichlorvos, an organophosphate pesticide, on the gill and liver tissues in Cirrhinus mrigala by light microscopy after exposing the fishes to sublethal concentrations (0.91 and 1.82 ppm) of dichlorvos for 10 days and observed hyperplasia, desquamation, and necrosis of epithelial cells, epithelial lifting, oedema, lamellar fusion, collapsed secondary lamellae, curling of secondary lamellae and aneurism in the secondary lamellae in gill tissues exposed to dichlorvos. Hepatic lesions in the liver tissues of fishes exposed to dichlorvos were characterized by cloudy swelling of hepatocytes, congestion, vacuolar degeneration, karyolysis, karyohexis, dilation of sinusoids and nuclear hypertrophy.

Tripathi et al., (2011) investigated the effects of the persistent environmental contaminant lindane, on the liver of a teleost fish Catla catla and reported that liver responses were rapid after exposure to 1.2% lindane for 30 days and the severity of the hepatocytic alterations was prominent and those changes in hepatocyte ultrastructure could have a wider relevance for ecotoxicology, as they are correlated with the survival capacity of the fish.

Ram Nayan Singh, (2012) was conducted a study to assess the histopathological damage of kidney in common carp, Cyprinus carpio after sub lethal exposure to dimethoate. In a short term (96 hr) study healthy juveniles of common carp were exposed to 0.96 mg/l of dimethoate (60% of 96 hr LC50) and observed remarkable changes in the kidney of exposed individuals in their histology in comparison to control. Observed prominent changes include shrinkage of glomerulus, and dilation of tubular lumen, vacuolization, desquamation, hydropic swelling and hyaline degeneration of tubular epithelium. Cyst formation and hemorrhage also observed in certain specimens and finally concluded that duration of exposure appears to have profound effect on kidney as with increasing duration of exposure histopathological damages become more severe.

Few reports of histological changes due to pesticides exposure on common carp are available (Neskovic, et al., 1996; Cengiz, 2006; Salvo et al., 2008, Ram Nayan Singh, 2012). But the literature on phorate toxicosis on the commercially important fish, Cyprinus carpio is extremely sparse. Moreover, no comparative studies are available on the acute and chronic toxicity of phorate, an organophosphate insecticide which is used widely in agricultural fields on different crops. Thus the present work is an effort to assess the impact of acute and chronic toxicity of phorate on the histology of gill, liver, muscle, kidney and brain of the common carp, *Cyprinus carpio*. From the present observations different cellular and subcellular
RESULTS

The structure of the gill of normal control Cyprinus carpio fish is composed of primary gill lamellae and secondary gill lamellae with well marked interlamellar spaces. Primary gill lamellae consisted of cartilaginous skeletal structure filament, multilayered epithelium and vascular system. Numerous secondary lamellae were lined up along both sides of primary lamella. Secondary gill lamella was constituted of epithelial cells supported by pillar cells (Fig I).

On exposure for a period of 1 day to acute toxicity of phorate no significant pathological changes were observed in the gills of the fish Cyprinus carpio except indications of the initiation of degenerative changes (Fig Ia). Further on exposure for a period of 4 days to acute toxicity of phorate, the pathological changes observed in the gills of the fish Cyprinus carpio were structural degeneration of primary gill lamellae, secondary gill lamellae and pillar cells along with epithelial lifting and desquamation. There was hypertrophy in the gill lamellae, erosion of surface epithelial cells and loss of lamellar structures in the gill due to atrophy of the gill lamellae (Fig Ib).

On exposure for a period of 1 day to chronic toxicity of phorate, mild degenerative changes were observed in the gill lamellae of the fish Cyprinus carpio. There was mild degree of hypertrophy in the epithelial cells of the gill lamellae but the primary and secondary gill lamellae were distinct with interlamellar spaces. (Fig Ic). On exposure for a period of 7 days to chronic toxicity of phorate there was a further damage in the structure of the gill. The primary and secondary lamellae of the gill showed epithelial hyperplasia and hypertrophy. Atrophy and swelling at the base with desquamation of the hyperplastic epithelia in the secondary gill lamellae was observed. Significant hemorrhagic condition was also noticed at the base of the primary gill lamellae (Fig Id). On exposure for a period of 15 days there was a further damage in the gill structure. Heavy swellings in the primary and secondary gill lamellae exhibited atrophy at the base with desquamation of the hyperplastic epithelia. Significant aneurism was also noticed in the gill lamellae. Significant damage in the structure of filament, primary and secondary lamellae of the gills with epithelial necrosis and desquamation was observed (Fig Ie). On exposure for a period of 30 days further degeneration in gill structure was observed with significant aneurism. Severe degeneration in the filament, primary and secondary lamellae of the gills and cytoplasmic vacuolization along with the
epithelial necrosis and desquamation was observed. The lamellar structure was lost with cytoplasmic vacuolization and erosion of gill epithelial cells (Fig If).

The structure of the normal liver of the control fish consists of continuous mass of cells called hepatocytes. The hepatocytes form a rather cord-like pattern and these cords are arranged around tributaries of the hepatic vein. The liver cells are large in size, polygonal in shape with homogenous granular cytoplasm and either eccentric or centrally located distinct nuclei. Each cord of the liver was separated by the thick wall of the peripheral cells (Fig II).

On exposure for a period of 1 day to acute toxicity of phorate mild degenerative changes were observed in the liver of the fish Cyprinus carpio, but the structure of the liver was distinct (Fig IIa). Further on exposure for a period of 4 days to acute toxicity of phorate, the pathological changes observed in the liver of the fish Cyprinus carpio were severe degree atrophy of the liver cords, degeneration of hepatocytes, and cytoplasmic disintegration. Cloudy swelling of hepatocytes, nuclear hypertrophy and nuclear degeneration were also noticed along with focal necrosis (Fig IIb). The liver was mostly disrupted due to the rupture of the cell membranes of the hepatocytes.

On exposure for a period of 1 day to chronic toxicity of phorate, no significant changes were observed in the structure of the liver of the fish Cyprinus carpio (Fig IIc). After 7 days of exposure to chronic toxicity of phorate some degenerative changes like nuclear hypertrophy, vacuolization in the hepatic cells and karyolysis were observed in the liver of the fish. The parenchymatous tissue was disrupted the liver cords were seen disarranged. Focal necrotic changes were seen in the liver cords with the degeneration of the hepatocytes (Fig IIId). On exposure for a period of 15days, the disintegration of liver cords was observed. Focal necrosis, degeneration of hepatocytes and karyolysis with widespread vacuolar appearance was noticed in the liver of the fish. Few hepatocytes lost their polygonal shape as they were hypertrophied and the cell membranes of some hepatic cells found to be degenerated (Fig IIe). On exposure for a period of 30 days to chronic toxicity of phorate, further increase in the structural degeneration with increasd vacuolization was observed. A severe degree atrophy of the liver cords and cytoplasmic disintegration appeared. The liver was disrupted due to the rupture of the cell membranes of the hepatocytes in the liver of the fish Cyprinus carpio (Fig IIIf).

The structure of the muscle of the normal control fish consist compactly packed muscle fibers with definite intermuscular spaces. There was no splitting in muscle fibers. The intermuscular spaces appeared to be filled with viscous fluid. Round to spindle shaped nuclei
were found distributing all over the bundle length with occasional hyper chromatia (Fig III).

On exposure for a period of 1 day to acute toxicity of phorate, splitting of muscle fibers followed by thinning of muscle fibers was observed in the muscle of the fish Cyprinus carpio. The muscle fibers exhibited longitudinal splitting with pyknotic nuclei (Fig IIIa). On exposure of for a period of 4 days, there observed a further increase in the longitudinally splitting of muscle fibers with pyknotic nuclei and cellular degeneration. Karyolysis seems to have occurred as granular debris. The isolation of muscle fibers from one and another was noticed with necrotic changes(Fig IIIb).

On exposure for a period of 1 day to chronic toxicity of phorate, mild degenerative changes were observed in the structure of the muscle of the fish Cyprinus carpio. The muscle fibers exhibited longitudinal splitting with cellular degeneration (Fig IIIc). On further exposure for a period of 7 days, isolation of muscle fibers followed by thinning of muscle fibers was observed. The muscle fibers exhibited longitudinal splitting with pyknotic nuclei. Cellular degeneration and increasing cytoplasmic vacuolization were also noticed (Fig IIIId). On exposure for a period of 15 days to chronic toxicity of phorate, further splitting in the muscle fibers followed by thinning of muscle fibers was observed. The muscle fibers exhibited longitudinal splitting with pyknotic nuclei. Degeneration of muscle fibers, nuclei and cellular necrosis were also noticed (Fig IIIe). On exposure for a period of 30 days, further degeneration of muscle was observed. There was heavy fibrilization with the loss of total muscular integrity and appearance of muscle fibers. The nuclei become highly pyknotic and scattered without any proper organization. The structure of the muscle was lost with cellular degeneration and cytoplasmic vacuolization in the fish Cyprinus carpio (Fig IIIf).

The structure of normal control kidney of the fish Cyprinus carpio consists of a clear Bowman’s capsule, glomerulus and renal tubules. The glomerulus, a cluster of capillaries surrounded by the Bowman’s capsule in the kidney (Fig IV).

On exposure for a period of 1 day to acute toxicity of phorate, compared to control, some structural changes that were observed in the kidney of the fish Cyprinus carpio are contraction in the glomerulus renal tubule and expansion of space inside the Bowman’s capsule. Initiation in the degeneration of hematopoietic tissue was also noticed (Fig IVa). On exposure for a period of 4 days to acute toxicity of phorate, further increase in the glomerular shrinkage and dilation in Bowman’s capsule was observed. The other pathological changes observed are appearance of periglomerular space, formation of pyknotic nuclei in the
hematopoietic tissue, necrosis in tubular epithelium, destruction in the renal tubules and vacuolation in the cytoplasm. Complete damage in the structural integrity of the kidney was noticed (Fig IVb).

On exposure for a period of 1 day to chronic toxicity of phorate, certain changes observed in the structure of the kidney of the fish Cyprinus carpio were glomerular Shrinkage and expansion of space inside the Bowman’s capsule. Nuclear hypertrophy in tubular epithelium cells with cloudy swelling of epithelial cells of renal tubules was observed. Cytoplasmic vacuolization with cellular degeneration was also noticed (Fig IVc). On exposure of the fish for a period of 7 days, some degenerative changes observed in the normal structure of the kidney, shrunken Glomerulus, dilated Bowman’s capsule, destruction in the tubules, vacuolation in cytoplasm and loss of architecture were observed. Necrosis in tubular epithelium and degeneration in Bowman’s capsule were seen (Fig IVd). Further on exposure of the fish Cyprinus carpio for a period of 15 days to chronic toxicity of phorate, some more degenerative changes were observed. Degeneration of glomerulus, increase in periglomerular space and degeneration in Bowman’s capsules were observed. Loss of cell architecture with increased vacuolation in the cytoplasm of the kidney was noticed (Fig IVe). After the exposure period of 30 days, reorganized dilated Bowman’s capsule, indistinct capillary structure, shrunken Glomerulus, tubular destruction, vacuolation in the cytoplasm, a clear increase in periglomerular space, loss of cell architecture and degeneration of cell nuclei were observed (Fig IVf).

The structure of normal brain of the control fish consists of clear neural cells with distinct nuclei. There was no discoloration, no significant lesion and any morphological change in the brain of control fish (Fig V). On exposure for a period of 1 day to acute toxicity of phorate, compared to control, mild degenerative changes were observed in the neural cells of the brain of fish Cyprinus carpio. Initiation of the degeneration of neural cells and structural degeneration was observed (Fig Va). On exposure for a period of 4 days to acute toxicity of phorate, further increase in the structural damage was observed. Necrosis of neurons, intracellular edema and congestion of neural cells were noticed. Degeneration of neural cells with the cytoplasmic vacuolization was observed (Fig Vb).

On exposure for a period of 1 day to chronic toxicity of phorate, certain degenerative changes observed in the in the structure of the brain of the fish Cyprinus carpio. Necrosis of neurons and formation of minor vacuoles with degeneration of neural cells and distended
sinusoids were observed. Significant hemorrhage also noticed at some places (Fig Vc). Further on exposure for a period of 7 days to chronic toxicity of phorate, significant changes were observed in the structure of the brain of the fish compared to the fish brain at day 1. Further structural damage, necrosis in neurons, intracellular edema and pycnotic nucleus were observed (Fig Vd). Further on exposure of the fish Cyprinus carpio for a period of 15 days to chronic toxicity of phorate, some more degenerative changes were observed. Increase in necrosis of neurons, cytoplasmic vacuolization observed. Severe lesions were also noticed in the brain of the fish (Fig Ve). After the exposure period of 30 days, reorganized neuronal tissue with indistinct nuclei was observed in the brain of the fish Cyprinus carpio (Fig Vf).

DISCUSSION

Histopathological investigations on different tissues of fish are valuable tools for toxicology studies and monitoring water pollutions. The histopathological investigations can provide information about the health and functionality of organs. Tissue injuries and damages caused by the pesticides in the organs of the fish 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 of fish exposure to pesticides.

In the present study, it is clearly indicated that the phorate has induced pronounced pathological changes in the gill, liver, muscle, kidney and brain of the fish Cyprinus carpio exposed to acute and chronic toxicity of phorate (Fig Ia to Vf). Various histopathological responses during the acute and chronic toxicity of pesticides could bring a relationship between the level of accumulation of the pesticide and to the various physiological and biochemical activities of the animal.

In the present study on exposure to acute and chronic toxicity of phorate relative to controls some degenerative changes were observed in the gill, liver, muscle, kidney and brain of the fish, Cyprinus carpio. In the gills degenerative changes were observed by epithelial hyperplasia and hypertrophy, atrophy and swelling at the base with desquamation of the hyperplastic epithelia in the secondary gill lamellae, erosion of surface epithelial cells, hemorrhagic conditions at the base of the primary gill lamellae, heavy swellings in the primary and secondary gill lamellae, significant aneurism in the gill lamellae, epithelial necrosis and desquamation and cytoplasmic vacuolization; in the liver by atrophy of the liver cords, cloudy swelling of hepatocytes, nuclear hypertrophy and nuclear degeneration in the
hepatocytes and vacuolization in the hepatic cells; in the muscle by splitting and isolation of muscle fibers, pyknotic nuclei, cellular necrosis and fibrilization of the muscle; in the kidney by shrunken glomerulus, destruction of renal tubules, dilation in Bowman’s capsule, increase in periglomerular space, necrosis in tubular epithelium and destruction in the renal tubules and in the brain by necrosis of neurons, intracellular edema and congestion in neural cells in the brain of the fish *Cyprinus carpio*.

These histopathological responses of the fish *Cyprinus carpio* exposed to acute and chronic toxicity of phorate in the present study reveal the degree of damage caused by this pesticide to the different tissues of the fish. The extent of damage caused by phorate to all the organs of the fish is less in chronic toxicity exposed fish than in acute toxicity exposed fish and the degenerative changes that were occurred in the gills, liver, muscle, kidney, and brain of the fish were progressive over the period of exposure to the acute and chronic toxicity of phorate suggest that the histopathological responses are depend on the concentrations of pesticides and the length of the period of fish exposure to pesticides.

Gills are generally considered as good indicator of water quality (Rankin et al., 1982) and being models for studies of environmental impact (Mallat, 1985; McKim and Erickson, 1991; Wenderlaar Bonga and Lock, 1992), since the gills are the primary route for the entry of pesticide. Fish gills come into immediate contact with the environment. In the present study, hyperplasia and hypertrophy of the epithelial cells, epithelial lifting, lamellar disorganization, lamellar aneurysm, rupture of the lamellar epithelium, rupture of pillar cells and necrosis were observed due to phorate toxicosis. Similar type of pathological changes were observed by many researchers on exposure to different organophosphorus pesticides.

De Silva and Samayawardhena, (2002) observed irregular appearance of gill lamellae, increased vacuolation in epithelial cell, lamellar fusion and complete destruction of gill lamellae in Poecilia reticulate exposed to chlorpyrifos. Cristina et al., (2008) observed lesions like epithelial ruptures, secondary gill lamellae fusion and hyperplasia of branchial epithelium in Carassius auratus gibelio after acute exposure to malathion. Similar type of pathological changes were also reported in Gambusia affinis due to long-term chronic toxicity of malathion (Cengiz and Unlu, 2003) and epithelial hyperplasia, aneurism, epithelial necrosis, desquamation, epithelial lifting, oedema, shortening of secondary lamellae and lamellar fusion in Cirrhinus mrigala exposed to dichlorvos (Vel muralugan et al., 2009). Sandipan Pal et al., (2012) reported hyperplasia and hypertrophy of gill epithelium, blood
congestion, dilation of marginal channel, epithelial lifting, lamellar fusion, lamellar disorganization, lamellar aneurysm, rupture of the lamellar epithelium, rupture of pillar cells and necrosis in the gill of common carp, Cyprinus carpio, intoxicated with sub-lethal concentrations of chlorpyrifos pesticide for a period of 14 days.

The damage occurred to the secondary gill lamellae with light precipitation of mucous and exfoliated nuclei, splitting of muscle fibers in the fish at day 1 of exposure to acute and chronic toxicity of phorate indicate that most of the pesticides affect the organ systems during the initial period of exposure. These changes may be a part of defense mechanism. The secondary gill lamellar changes in the gill of the fish might have occurred due to the failure of gaseous changes across the respiratory epithelium on exposure to the acute and chronic toxicity of phorate. It can be speculated that pathological alterations like hyperplasia of epithelial cells, epithelial lifting and lamellar fusion may increase the space of contact of toxicants with the vascular system of the gill, resulting in impairment of respiration as well as fish health. As gills are crucial organs for their respiratory, osmoregulatory and excretory functions in fish, the cellular damage in the gills of the fish Cyprinus carpio induced by phorate toxicity might also impaired the osmoregulatory function of the fish evidenced from the decreased oxidative metabolism. Aneurism was observed at day 4 on exposure to acute toxicity of phorate and at day 15 on exposure to acute toxicity of phorate. It occurs due to collapse of pillar cells in the secondary lamellae and rupture of blood vessel, releasing large quantities of blood that push the lamellar epithelium outward. Rupture of the lamellar epithelium, rupture of pillar cells and necrosis are the direct deleterious effects induced by phorate exposure.

The liver is the main organ for detoxification (Dutta et al., 1993) that suffers serious morphological alterations in fish exposed to pesticides (Rodrigues and Fanta, 1998). Alterations in the liver may be useful as a marker that gives prior indication of pathological alterations on exposure to environmental stressors. After exposure to acute and chronic toxicity of phorate congestion, cloudy swelling of hepatocytes, disarrayed structure of liver cords, nuclear hypertrophy and degeneration and focal necrosis were observed. The histological changes noticed in the exposed fish were supported by the metabolic disorders like the depletion in total carbohydrate observed in it. The biochemical disorders observed in the liver of the fish exposed to acute and chronic toxicity of phorate could be due to its gradual structural disorganization.
Similar pathological changes like in the present study were observed by several investigators in the liver of fish on exposure to different pesticides. Fanta et al., (2003) observed cloudy swelling, focal necrosis, atrophy and vacuolization in the Corydoras paleatus exposed to methyl parathion. Sarkar et al. (2005) reported hyperplasia, vacuolation, disrupted hepatocytes, focal coagulative necrosis, disorganized hepatic canaliculi in *Labeo rohita* exposed to cypermethrin. Cengiz and Unlu, (2006) observed hepatic lesions in the liver tissue of fish Gambusia affinis exposed to deltamethrin were hypertrophy of hepatocytes, increase of Kupffer cells, circulatory disturbances, focal necrosis, fatty degeneration, nuclear pycnosis and narrowing of sinusoids. Sandipan Pal et al., (2012) observed nuclear and cellular hypertrophy, cellular atrophy, irregular contour of cells and nucleus, cytoplasmic vacuolation, cytoplasmic and nuclear degeneration, cellular rupture, pyknotic nucleus, necrosis and melanomacrophages aggregations in the liver of common carp, Cyprinus carpio, intoxicated with sub-lethal concentrations of chlorpyrifos pesticide for a period of 14 days.

The muscle of the fish Cyprinus carpio showed different pathological changes on exposure to acute and chronic toxicity of phorate such as splitting and isolation of muscle fibers, pyknotic nuclei, cellular necrosis and fibrilization and inflammation in muscle fibers. The metabolic disorders observed in the muscle were supported by the progressed degenerative changes in it on exposure to acute and chronic toxicity of phorate. The pathological changes that were observed suggest that significant concentration of pesticide accumulation in this organ. These changes affect the contractile ability and cause for disfunctioning of muscle fibers.

Kidney serves as a major route of excretion of metabolites of xenobiotics, and receives the largest proportion of postbranchial blood and therefore, it is more likely to undergo histopathological alterations under pesticide stress (Ortiz et al., 2003). Therefore renal lesions might be expected to be good indicators of environmental pollution (Ortiz et al. 2003). The histological changes such as hypertrophied epithelial cells of renal tubules, pycnotic nuclei in tubular epithelium, contraction of the glomerulus and expansion of space inside the Bowman’s capsule were observed in the kidney tissue of fish Cyprinus carpio after exposure to acute and chronic toxicity of phorate. The cellular damage which occurred in the kidney due to the toxicity of phorate impairs the osmoregulatory function which was evident from the impaired oxidative metabolism as the kidney is not only the excretory organ but also functions as osmoregulatory organ of the fish. The histological changes observed in the kidney of the fish *Cyprinus carpio* exposed to acute and chronic toxicity of phorate were supported by the
metabolic disorders observed in this organ in the present study.

Several investigators have been found histological alterations earlier at the level of the tubular epithelium and glomerulus in the kidney of this fish. Dilation of tubules, necrotic changes characterized by karyorrhexis and karyolysis at the nuclei of affected cells were observed in the fish *Labeo rohita* exposed to hexachlorocyclohexane (Das and Mukherjee, 2000). Tilak et al. (2001) reported severe necrosis, cloudy swelling in the renal tubules, cellular hypertrophy, granular cytoplasm, vacuolization in kidney tissues of *Ctenopharyngodon idellus* exposed to fenvalerate. Cengiz (2006) observed degeneration in the epithelial cells of renal tubule, pycnotic nuclei in the hematopoietic tissue, dilation of glomerular capillaries, degeneration of glomerulus, intracytoplasmatic vacuoles in epithelial cells of renal tubules with hypertrophied cells and narrowing of the tubular lumen are observed in the kidney tissues of fish *Cyprinus carpio* exposed to deltamethrin. Ram Nayan Singh, (2012) observed shrinkage of glomerulus, dilation of tubular lumen, vacuolization, desquamation, hydropic swelling and hyaline degeneration of tubular epithelium cyst formation and hemorrhage in certain specimens of the kidney of common carp, *Cyprinus carpio* after sub lethal exposure to dimethoate and reported that duration of exposure appears to have profound effect on kidney as with increasing duration of exposure histopathological damages become more severe.

Brain is the controlling centre for all functions and movements in the body organisms like fish serving as a relay station. In the present study hyperplasia, edema, necrosis and increase in brain cells were some of the histological changes observed in the brain of the fish *Cyprinus carpio* exposed to acute and chronic toxicity of phorate.

The pesticides present in water reaches the fish body through water taken in with food, mucosa of the mouth or gills and they may reach liver, muscle, kidney and brain through blood circulation. Various regions in fish brain are concerned with many functions. The impairment of tissue of a region in the brain by these pathological changes may lead into the curtailment of the particular function in fishes. This alters the physiological and behavioural functions of the fish. This is evident by an inhibition of AChE activity and impairment in the fish behavior in the present study. This indicates that phorate exerted a neurotoxic effect and impairment of neural conductivity in the central and peripheral nervous system.

observed vascular dilation in fish brain on exposure to 2, 4-D and endosulfan respectively. Pugazhvendan et al., (2009) observed loss of differentiation in brain cells, scatterly arranged cells and severe necrosis in the brain of cells in Ophiocephalus punctatus exposed to malathion pesticide.

Thus the histological changes that were taken place in the present study, at the initial period of exposure in the organs of the fish on exposure to acute and chronic toxicity of phorate might be a part of defense mechanism. On prolonged exposure due to further accumulation of phorate in the organs of the fish, it caused destruction in the organ structures. The slight structural reorganization of the gills, liver, muscle, kidney, and brain of the fish observed at day 30 of exposure to chronic toxicity of phorate gives support to some extent that the ability of the fish to resist the sublethal stress and in repair of the damage caused to the organs by enhancing the protein synthetic potentials and other associated activities of the cell. Probably the fish could excrete or chelated the accumulated phorate over the time of exposure, there by the toxic effect of it might have been gradually decreased. The degree of destruction in the organs of the fish appeared to be linearly proportional to the period of exposure (Ram Nayan Singh, 2012; Sandipan Pal et al., 2012).

On overall assessment, on exposure to acute and chronic toxicity of phorate, though initially caused a mild damage to the organs of the fish but further exposure to acute toxicity of phorate it caused irreversible damage to the organs of the fish. On prolonged to exposure chronic toxicity of phorate fish could develop enough resistance and replenish the loss by activating the energy cycles. Thus the changes induced by acute and chronic toxicity of phorate in the structure and morphology of the organs of the fish cyprinus carpio are not only dependent on the concentration of the pesticide but also on the length of the exposure period. Frequency and intensity of tissue lesions depend on the concentrations of pesticides and the length of the period of fish exposure to pesticides.
LEGEND FOR FIGURES

PLATE - 1

**Fig: I.** The normal architecture of the control fish Gill tissue showing primary lamellae (PL), secondary lamellae (SL), epithelial cells (EC), lamella (L), filament (F), chondrocytes (C) and pillar cells (PC) with well marked inter lamellar spaces with lower magnification (10X) and Higher magnification (40X).
Fig: Ia. The Gill of the fish exposed to acute toxicity of phorate for 1 day showing primary lamellae (PL), secondary lamellae (SL), epithelial cells (EC), lamellar filament (LF) and pillar cells (PC) with mild degenerative changes in normal cytoarchitecture with lower magnification (10X) and Higher magnification (40X).

Fig: Ib. The Gill of the fish exposed to acute toxicity of phorate for 4 days showing structural degeneration (SD) in primary gill lamellae (PL), secondary gill lamellae (SL), filament (F) and pillar cells (PC) along with the degenerative changes (DC) such as epithelial lifting (EL), desquamation (DN) epithelial lifting and desquamation (ELD) with lower magnification (10X) and Higher magnification (40X).
Fig: Ic. The Gill of the fish exposed to chronic toxicity of phorate for 1 day showing structural changes (SC) in primary lamellae (PL), secondary lamellae (SL) and filament (F) with mild degenerative changes (DC) in normal cytoarchitecture with lower magnification (10X) and Higher magnification (40X).

Fig: Id. The Gill of the fish exposed to chronic toxicity of phorate for 7 days showing further degenerative changes (DC) in primary gill lamellae (PL), secondary gill lamellae (SL), filament (F) and submucosa (SM) along with epithelial lifting (EL), epithelial hyperplasia (EH) and epithelial lifting and desquamation (ELD) with lower magnification (10X) and Higher magnification (40X).

Fig: Ie. The Gill of the fish exposed to chronic toxicity of phorate for 15 days showing degeneration of lamella (DL), filament (F) and primary gill lamellae (PL) with aneurism (A), epithelial necrosis (EN), epithelial necrosis and desquamation (END) & epithelial lifting and desquamation (ELD) with lower magnification (10X) and Higher magnification (40X).

Fig: If. The Gill of the fish exposed to chronic toxicity of phorate for 30 days showing degenerative changes (DC) in primary gill lamellae (PL), secondary gill lamellae (SGL) and lamellar filament (LF) with necrotic changes (NC), aneurism (A), epithelial lifting (EL) desquamation (DN), epithelial lifting and desquamation (ELD), structural degeneration (SD) and degeneration in primary lamellae (DPL) with lower magnification (10X) and Higher magnification (40X).
LEGEND FOR FIGURES

PLATE - 4

Fig: II. The normal architecture of the control fish liver tissue showing continuous mass of polygonal cells called hepatocytes (H), eccentric or centrally located distinct nuclei (N) and central vein (CV) with lower magnification (10X) and higher magnification (40X).

LEGEND FOR FIGURES

PLATE - 5

Fig: IIa. The liver of the fish exposed to acute toxicity of phorate for 1 day showing
hepatocytes (H), nuclei (N) and central vein (CV) with congestion (C) and mild degenerative changes (DC) in normal cytoarchitecture with lower magnification (10X) and Higher magnification (40X).

**Fig: IIb.** The liver of the fish exposed to acute toxicity of phorate for 4 days showing structural degenerative changes (SDC) such as cloudy swelling of hepatocytes (CSH) nuclear hypertrophy (NH), nuclear degeneration (ND), pycnotic nucleus (PN), structural degeneration (SD) and vacuolization with focal necrosis (FN) and cytoplasmic vacuolization (CVN) with lower magnification (10X) and Higher magnification (40X).

**LEGEND FOR FIGURES**

**PLATE - 6**

**Fig: IIc.** The liver of the fish exposed to chronic toxicity of phorate for 1 day showing hepatocytes (H), nuclei (N) and central vein (CV) with initiation of degenerative changes
(DC) in normal cytoarchitecture with lower magnification (10X) and Higher magnification (40X).

**Fig: IId.** The liver of the fish exposed to chronic toxicity of phorate for 7 days showing nuclei (N), degenerative changes (DC) such as degeneration of hepatocytes (DH) nuclear hypertrophy (NH), karyolysis (KL) with focal necrosis (FN), structural degeneration (SD), cytoplasmic vacuolization (CV) and formation of vacuoles (V) with lower magnification (10X) and Higher magnification (40X).

**Fig: IIe.** The liver of the fish exposed to chronic toxicity of phorate for 15 days showing nuclei (N), degenerative changes (DC) such as degeneration of hepatocytes (DH) karyolysis (KL) with focal necrosis (FN), cytoplasmic vacuolization (CV) and formation of vacuoles (V) with lower magnification (10X) and Higher magnification (40X).

**Fig: IIIf.** The liver of the fish exposed to chronic toxicity of phorate for 30 days showing hepatocytes (H), structural degenerative changes (SDC) such as degeneration of hepatocytes (DH), degeneration of nucleus (DN) and cytoplasmic vacuolization (CV) with lower magnification (10X) and Higher magnification (40X).
LEGEND FOR FIGURES

PLATE – 7

Fig: III. The normal architecture of the control fish muscle tissue showing compactly packed muscle fibers (PMF), intermuscular spaces (IMS) and round to spindle shaped nucleus (SSN) with lower magnification (10X) and higher magnification (40X).

LEGEND FOR FIGURES

PLATE – 8

Fig: IIIa. The muscle of the fish exposed to acute toxicity of phorate for 1 day showing packed muscle fibers (PMF), nuclei (N) and degenerative changes (DC) such as initiation of structural changes (SC), splitting of muscle fibers (SMF) and increasing intermuscular spaces.
(IMS) with lower magnification (10X) and Higher magnification (40X).

**Fig: IIIb.** The muscle of the fish exposed to acute toxicity of phorate for 4 days showing nuclei (N), structural degenerative changes (SDC), necrotic changes (NC), cellular degeneration (CD) and increasing inter muscular spaces (IMS) with lower magnification (10X) and Higher magnification (40X).

**LEGEND FOR FIGURES**

**PLATE – 9**

**Fig: IIIc.** The muscle of the fish exposed to chronic toxicity of phorate for 1 day showing packed muscle fibers (PMF) and inter muscular spaces (IMS) with mild degenerative changes (DC) in normal cytoarchitecture with lower magnification (10X) and Higher magnification (40X).
Fig: IIId. The muscle of the fish exposed to chronic toxicity of phorate for 7 days showing nuclei (N), degenerative changes (DC) such as splitting of muscle fibers (SMF), pycnotic nucleus (PN), cellular degeneration (CD) and degeneration of muscle fibers (DMF) with lower magnification (10X) and Higher magnification (40X).

Fig: IIIe. The muscle of the fish exposed to chronic toxicity of phorate for 15 days showing degenerative changes (DC) such as splitting of muscle fibers (SMF), necrotic changes (NC), degeneration of nucleus (DN), cellular degeneration (CD) and degeneration of muscle fibers (DMF) with lower magnification (10X) and Higher magnification (40X).

Fig: IIIf. The muscle of the fish exposed to chronic toxicity of phorate for 30 days showing further increase in the degenerative changes (DC) such as splitting of muscle fibers (SMF), necrotic changes (NC), degeneration of nucleus (DN), cellular degeneration (CD) and degeneration of muscle fibers (DMF) with lower magnification (10X) and Higher magnification (40X).
PLATE – 10

**Fig: IV.** The normal architecture of the control fish Kidney tissue showing a clear Bowman's capsule (BC), glomerulus (G), renal tubules (RT), lumen of the renal tubules (L) nuclei of renal tubules (N) epithelial cells renal tubules (ERT) and hematopoietic tissue (HT) with lower magnification (10X) and Higher magnification (40X).

**LEGEND FOR FIGURES**

PLATE – 11

**Fig: IVa.** The kidney of the fish exposed to acute toxicity of phorate for 1 day showing congestion, initiation of degenerative changes (DC) in Bowman’s capsule (BC) and hematopoietic tissue with lower magnification (10X) and Higher magnification (40X).
**Fig: IVb.** The kidney of the fish exposed to acute toxicity of phorate for 4 days showing degenerative changes (DC) such as glomerular shrinkage (GS), expansion of space inside the Bowman’s capsule (ES), degeneration of Bowman’s capsule (BC) necrotic changes (NC) and cytoplasmic vacuolization (CV) with lower magnification (10X) and Higher magnification (40X).

**LEGEND FOR FIGURES**

**PLATE – 12**

**Fig: IVc.** The kidney of the fish exposed to chronic toxicity of phorate for 1 day showing initiation of degeneration of glomerulus (DG) and Bowman’s capsule (BC), shrunken glomerulus (SG), increase in periglomerular space (PS), nuclear hypertrophy in tubular
epithelium cell (NH) and vacuolization (V) with lower magnification (10X) and Higher magnification (40X).

**Fig: IVd.** The kidney of the fish exposed to chronic toxicity of phorate for 7 days showing structural degenerative changes (SDC) such as shrunken glomerulus (SG), degeneration of Bowman’s capsule (DBC), necrosis in tubular epithelium (NT) and cytoplasmic vacuolization (CV) with lower magnification (10X) and Higher magnification (40X).

**Fig: IVe.** The kidney of the fish exposed to chronic toxicity of phorate for 15 days showing degenerative changes (DC) such as degeneration of glomerulus (DG), increase in periglomerular space (PGS), degeneration of Bowman’s capsule (DBC), degeneration of renal tubule (RT) and increasing cytoplasmic vacuolization (CV) with lower magnification (10X) and Higher magnification (40X).

**Fig: IVf.** The kidney of the fish exposed to chronic toxicity of phorate for 30 days showing degenerative changes (DC) such as shrunken glomerulus (SG), degeneration of Bowman’s capsule (DBC), necrosis in tubular epithelium (NT) and cytoplasmic vacuolization (CV) with lower magnification (10X) and Higher magnification (40X).

**LEGEND FOR FIGURES**
PLATE – 13

**Fig: Va.** The normal architecture of the control fish Brain tissue showing clear hippocampus (HI), neural cells with distinct nuclei with lower magnification (10X) and Higher magnification (40X). There was no discoloration, no lesion and any morphological change in the brain of control fish.

LEGEND FOR FIGURES

PLATE – 14

**Fig: Va.** The Brain of the fish exposed to acute toxicity of phorate for 1 day showing clear neural cells and distinct nuclei with mild degenerative changes (MDC) and mild structural damage (SD) in normal cytoarchitecture with lower magnification (10X) and Higher magnification (40X).
**Fig: Vb.** The Brain of the fish exposed to acute toxicity of phorate for 4 days showing nuclei (N), degenerative changes (DC) such as structural degeneration (SD) and congestion (C) with lower magnification (10X) and Higher magnification (40X).

**LEGEND FOR FIGURES**

**PLATE – 15**

**Fig: Vc.** The Brain of the fish exposed to chronic toxicity of phorate for 1 day showing nuclei (N), degenerative changes such as hemorrhage (He) distended sinusoids (DS) and minor vacoulation (MV) in normal cytoarchitecture with lower magnification (10X) and Higher magnification (40X).

**Fig: Vd.** The Brain of the fish exposed to chronic toxicity of phorate for 7 days showing nuclei (N), degenerative changes (DC) and structural degeneration (SD) with lower
magnification (10X) and Higher magnification (40X).

**Fig: Ve.** The Brain of the fish exposed to chronic toxicity of phorate for 15 days showing degeneration of neural cells and formation of vacuoles (V) with lower magnification (10X) and Higher magnification (40X).

**Fig: Vf.** The Brain of the fish exposed to chronic toxicity of phorate for 30 days showing degenerative changes (DC) such as degeneration of neural cells and structural damage (SD) with lower magnification (10X) and Higher magnification (40X).
PLATE - 15

Brain of the fish exposed to chronic toxicity
PLATE - 14
Brain of the fish exposed to acute toxicity of p
PLATE - 13
Control Brain
PLATE - 12
Kidney of the fish exposed to chronic toxicity of
PLATE - 11
Kidney of the fish exposed to acute toxicity of p
PLATE - 10
Control Kidney
PLATE- 9
Muscle of the fish exposed to chronic toxicity of
PLATE - 8
Muscle of the fish exposed to acute toxicity of
PLATE - 7
Control Muscle
PLATE - 6
Liver of the fish exposed to chronic toxicity of
PLATE - 5
Liver of the fish exposed to acute toxicity of p

Fig. Iia

Fig. Iib
PLATE - 4
Control Liver
PLATE - 3

Gill of the fish exposed to chronic toxicity of
PLATE- 2
Gill of the fish exposed to acute toxicity of phy
PLATE - 1
Control Gills

Fig. 1 10X

Fig. 1 40X