Histopathology in the Greek-histos (tissues) and pathos (Suffering) refers to the microscopic examination of tissue in order to study the manifestation of disease, specifically in clinical medicine, histopathology refers to the examination of a biopsy or surgical specimen by a pathologist after the specimen has been processed and histological sections have been placed onto glass slides. This is the most important tool of the anatomical pathologist in routine clinical diagnosis, of wounds, cancer, diabetes and other diseases.

Histology in a precise sense is the study of the cytoarchitectural change of the body, which envisage the anatomy and gives the insight into the functioning of tissues and organs. Thus histology is a structural science and serves to compliment the knowledge gained from the anatomy, physiology and pathology.

Histology gives the insight into the functioning of tissues and organs. In a precise sense it is the study of architectural change of the body which envisages the anatomy (Jayantha Rao, 1982). Histology, the study of microanatomy of specific tissues, has been successfully employed as a diagnostic tool in medical and veterinary sciences since the first cellular investigation carried out in nineteenth century (Virchow, 1858).

Histology is the study of sectioned as a thin slice, using a microtome. Histopathology is the microscopic study of diseased tissue and is an important tool of anatomical pathology. The trained scientists who perform the preparation of histological sections are known as histotechnicians, histology technicians (HT), histology technologists (HTL), and medical scientists, medical laboratory technicians or biomedical scientists. Their field of study is called histotechnology (Merck Source, 2002).

Exposure of animals to contaminated water also causes severe pathological changes at the tissues level. Histology is a structural science and serves to compliment the knowledge gained from the anatomy, physiology and pathology and it gives insight into the functioning of tissues and organs. A clear picture of cytoarchitectural changes produced during chemical intoxication can be assessed by histological studies. Histopathology helps in diagnosing the damages of the tissues of an animal subjected to toxic stress. 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).

Histology is the study of the microscopic anatomy of cells and tissues of plants and animals. It is performed by examining a thin slice (section) of tissue under a light microscope or electron microscope. The ability to visualize or differentially identify microscopic structures is frequently enhanced through the use of histological stains. Histology is an essential tool of biology and medicine has been successfully employed as a diagnostic tool in medical and veterinary science, first cellular investigations were carried out in the mid nineteenth century (Virchow, 1858).

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 or metal. Histopathological assessment throws light on the nature of tissue alternation and the extent of damage. This in turn indicates the toxic nature of the compound. Therefore, histology gives useful insight to the tissue lesions prove to the external manifestations of the deleterious effects of heavy metals (Jayantha Rao, 1982). 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. These studies may explain, to some extent, the tissue specificity of the toxicant and also many open new avenues in toxicology.

The examination and study of normal cells and tissues by microscopy is called histology or microscopic anatomy. The study of abnormal cells and tissues is histopathology (Aughey and Frye, 2001).

It is obvious that any chemical insult could cause pathological damage if it is taken beyond the safe permissible limits. The intensity of injury varies greatly among the tissues and cells of the some animals and more so among different animal groups. The advance effects of a chemical agent on any animal depends on three variables.

1. The vulnerability of individual tissues.
2. The mode of action of the agent.
3. The concentration of the agent.
However, the location of major damage may be determined by the mode of action of the chemical. Some of the chemicals, if present in the environmental media, exert their effect locally at the portal of entry, leading to damage to the external surface of the body or if they are ingested, through proximal portion of the gastrointestinal track will be affected.

Histology and cytology are concerned primarily with morphological characters of microscopic structures. This helps in understanding the chemistry of microscopic structure is termed histochemistry or cytochemistry, depending on whether the object of interest is the tissue or cell. 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 produced during the chemical intoxication can be traced by histopathological studies. Several workers reported on the pesticides and pointed out the architectural damage to brain, gill, liver, kidney, heart, lung, muscle, tests, intestine in various animals (Shukla et al., 2001, Glynn, 2003; Garg et al., 2004; Sivaiah, 2006; Jayasankar, 2007; Ravi Sekhar et al., 2011, 2012; Kavitha et al., 2013; Praveena and Jayantha Rao, 2013).

Results

Normal histology of mice liver

The liver of normal mice comprises of continuous mass of hepatic cells with cord like formation. The cells are large in size with more or less centrally placed nucleus and homogenous cytoplasm. There is no clear division of the hepatic cells into lobules. The hepatic cells are hexagonal in their nature. The bulk of the hepatic lobule comprised of polyhedral epithelial parenchyma cells containing round nuclei and a prominent nucleolus. A fine network of vascular capillaries, sinusoids running in between the parenchyma cells, the nucleus in hepatocytes consists one or more nucleoli were noticed (Plate 5.1; Figs. A&B).

Hepatic cells have many vital functions other than secretion of bilie. They play an important role in detoxification, serve as storage sites for some nutrients and are also involved in carbohydrate, protein and lipid metabolism.
Experimental mice liver

The mice exposed to sodium fluoride separately and in combination with Vit. C for 7 days and 30 days have shown remarkable changes when compared to control (Plates 5.2 – 5.5; Figs. C – K). These changes include – moderate cytoplasmic degenerative changes in hepatocytes, cellular degeneration, vacuoles, congestion, cellular disarray, nuclear fragmentation, nuclear degenerative changes, binucleated condition, pushing of nucleus to periphery of hepatocytes, severe necrosis in hepatocytes, (Plates 5.2 – 5.5; Figs. C – K). In 30 days sodium fluoride showed more changes compared to sodium fluoride+vit.C. The mice which received sodium fluoride and vitamin C in combination have exhibited recovery changes compared to the mice received NaF.

Normal histology of mice testis

The testis of control mice consisting of several seminiferous tubules. The seminiferous tubules are covered with connective epithelial layer. The seminiferous tubule under microscope showed spermatids, matured spermatozoa and seratoli cells. Interstitial cells (Leydig cells) were also observed in between the seminiferous tubules (Plate 5.6; Figs. L & M).

Mice testis under experimental condition

Mice exposed to sodium fluoride separately and in combination with vit. C for 7 and 30 days have shown several pathological lesions which include – clumped spermatozoa, degenerative changes in seminiferous tubules, increase of lumen, degeneration in theca albuginea, necrosis in seminiferous tubules, appearance of vacuoles in seminiferous tubules, scattered spermatids, degeneration of leydig cells and atrophy of seminiferous tubules (Plate 5.7 – 5.10; Figs. N – V). These changes are more in 30 days compared to 7days. Recovery was noticed in vit. C administered mice in both the cases.
Discussion

In the present investigation mice were exposed to sodium fluoride and vitamin C separately and in combination for 7 and 30 days. After stipulated time the tissues like liver, and testis were analysed for histopathological changes. Evidently the liver showed moderate to severe degenerative changes in cytoplasm of hepatocytes, nuclear degeneration, fragmentation of nuclei, binucleated condition, congestion, vacuoles and cellular disarray (Plates 5.2 - 5.5; Figs. C - K). These changes are responsible for alteration in biochemical parameters in the present investigation (Chapters II&III). Thus, the chemicals individually and in combination resulted the altered biochemical path ways in the present investigation.

Liver is the largest organ of the body comprising 2-3% of the total adult body weight, is primarily concerned with the metabolic activity of organisms (Sheila and Dooley, 1993). It is also the central site for the biotransformation of xenobiotic chemicals and therefore is involved in the detoxifying mechanism of the body. Liver is responsinble for detoxifying the chemical.

Several authors reported histopathological changes in liver in different animal models under pesticidal toxicity.

Hypertrophy of hepatocytes with pyknotic nuclei, vacuoles and hyalinization, hepatocytes with dilation of central vein in albino mice treated with carbosulfan (Ksheerasagar and Kaliwal, 2006).

Sarkar et al. (2005) reported as hyperplasia, disintegration of hepatic mass, focal coagulative necrosis ion Labeo rohita. In mice, Turki Al-Shaikh (2013), observed liver of necrosis of hepatic cells with pyknotic nuclei, disorganization of hepatic laminae, and dilation of sinusoids exposed to cypermethrin. Velisek et al. (2006) observed degeneration of hepatocytes in the periportal zones and affected hepatocytes and many small or one big vacuole in the cytoplasm in the liver of Oncorhynchus mykiss exposed to cypermethrin.

Congestion and disruption of sinusoids after oral administration of abamectin at 30 mg/kg for 30 days and 10 mg/kg for 210 days exposed to rat (Hany Kamal Abd-Elhady and Gamel Elsayed Abou-Elghar, 2013).
Hepatic lesion in the liver tissues of *Cirrhinus mrigala* exposed to fenvalerate were characterized by congestion, cloudy swelling of hepatocytes and focal necrosis (Velmurugan *et al.*, 2007). Liver showing blood streakes, fibrosis and vacuolated hepatocytes with pycnosis nucleus in *H. fossilis* treated with cypermethrin (Pratap Sing and Vandana Sing, 2008).

Rats were exposed to 0.5mg/kg and 1 mg/kg cadmium chloride for 3 days caused severe hepatic injury like hydropic degeneration of hepatocytes, granulation, bile duct proliferation (Udita Gubrelay *et al.*, 2004).

Ajay Kumar *et al.* (2014) observed blood congestion and dilated sinousoids and showing distorted central vein ,and congestion with dilation of sinusoids, accumulation of RBC in vein central in albino mice treated with 75.5 mg/kg b.w.,112.5 mg/kg bw Imidacloprid.

Swapnila Chouhan *et al.* (2010) reported showing hepatocytic vacuolation, cells showing pyknotic nuclei with ballooning of hepatocytes and karyolysis with NaF (50ppm). The liver of mice treated with 10, 20, 40 ppm NaF showed swellings, necrosis, and vacuolization of hepatocytes. Necrosis increased with rising doses of fluoride (Yusuf Ersan *et al.*, 2010).

Liver histopathology showed centrilobular necrosis, hepatic cell degeneration and necrosis with loss of nucleus. The administration of vitamin c attenuated sodium fluoride induced hepatotoxicity via noramalization of histopatopathological changes induced by sodium fluorde.

Pathomorphological changes were observed in the liver of mice, a similar, protective effect of vitamins against such changes was demonstrated in the male rats intoxicated with NaF (Barbara Stawiarska *et al.*, 2013). Such changes were demonstrated in the pancreas and lungs of male rats intoxicated with NaF (Stawiarska *et al.*, 2008). There is also evidence of F-induced oxidative stress in the testis and ovary as well as in other organs (Chinoy *et al.*, 2005).

Vitamin C ameliorated stannous chloride induced toxicity in the liver of male rabbits and improved liver architecture in stannous chloride treated rats has been reported (El-Demerdash *et al.*, 2005, Morsy *et al.*, 2010). This vitamin also helps the liver in the detoxification of toxic substances in the system, and the blood in fighting
infections (Pooja and Mishra Sunita, 2014). Thus, vitamins C could ameliorate the liver damage induced by malathion (Suna Kalender, 2010). Vit. C, a low molecular weight antioxidant, defends the cellular compartment against water-soluble oxygen nitrogen radicals (Jurczuk et al., 2007).

Mice testis also showed pronounced changes in the experimental animals in the present investigation. The changes include – degenerative changes in spermatids, clumped spermatozoa, increased area of lumen of seminiferous tubules, degeneration in leydig cells, atrophy of seminiferous tubules and fragmentation of seminiferous tubules (Plates 5.7 – 5.10; Figs. N – V). These changes have direct effect on certain enzymes responsible for androgens and the production of spermatozoa which were observed in the present investigation (Chapter – III).

Fluoride treatment is associated with testicular disorders, which may be due to induction of oxidative stress in reproductive organs along with possible adverse effects of fluoride on pituitary testicular axis. The detailed mechanism of fluoride treatment on the male reproductive system has not been elucidated and will be the subject of future experiments (Ghosh et al., 2002).

Alsayed Ali Mahran et al. (2011) observed positive reaction in the disorganized germ cells lining of the seminiferous tubules and the other testicular tissue damaged of their basement membranes (head arrow) treated with aluminum chloride.

Testis histopathology following F treatment revealed loss of spermatozoa, pyknosis, vacuolization, disorganization of germ cells, and atrophic Leydig cells, supporting the view that oxidative stress induced by F affected gonadal functions resulted in severe alterations in the histology of testis which disturbed the process of spermatogenesis (Chinoy et al., 2005).

Nahid Akhtar et al. (2009) observed increased interstitial space along with sloughing of germinal cells into the lumen that when the rats were exposed to a chlorpyrifos at the doses of 3, 6 and 9 mg kg-1d-1 for 90 days.

Ghosh et al. (2002) found edema between seminiferous tubules, vacuolization, and hyalinization in the tubules of the testes of rats exposed to α-cypermethrin. In addition, a decrease in luminal sperm and apparent dilation of
tubules, together with oxidative stress, has been associated with testicular damage from NaF and aluminium treated rats Mahita et al. (2011).

Manna et al. (2005) reported edematous fluid accumulation between the tubules and vacuole formation within the tubules in testes of rats exposed to deltamethrin. Necrosis in the connective tissue, degeneration of spermatids, atrophied seminiferous tubules and reduced lumen, atrophied spermatozoa, clumping of spermatozoa and necrosis in interstitial cells in mice testis exposed to monocrotophos and azadirachtin (Sivaiah, 2006).

Smita Tiwari and Pande (2011) reported on reproductive tissue damage in young male rat exposure of Sodium Fluoride (5, 20, 50 mg/kg b.w.) for 90days. Damaging effect on testicular histoarchitecture along with disfigured tubular structure was recorded along with histological change in other organs viz.-Epididymis, vasdeference seminal vesicle, and prostate gland. Even the epermatogenesis seemed to be arrested and clumping of spermatozoa reported.

Several investigators have also reported the effect of metals on testes, liver. (Fairoze Khattab, 2007; Arnab Bhattacharya, 2012; Hamid Reza Momeni, 2012) Ravi Sekhar et al. (2011) described combination of cypermethrin, sodium fluoride severe necrosis in seminiferous tubules increased lumen of seminiferous tubules, and necrotic changes in the theca albuginea when mice were exposed to cypermethrin and NaF and their combination.

Vitamin C reduced the toxicity of dichlorvos on the composition of the testis tissue the male rats (Dirican and Kalender, 2012) and Vitamins reduced the toxicity of the pesticide deltamethrin on the reproductive system in male mice (Turki Al-Shaikh, 2013) has been observed.

Treatment with Vitamin - C has shown clear histological, histometric, signs of recovery from NaF exposure-related deteriorations in germ line cells in mice indicating its ameliorative potentials in male sex related toxicology. Similar observations were reported by many authors regarding recovery studies in mice and rat (Chinoy et al., 2004; Khawaja Ahmad et al., 2012 and Mandava Rao and Rajendra Bhatt, 2012; Shweta Parihar et al., 2013).
Naresh Kumar et al. (2012) reported lack of differentiation and maturation of spermatocytes and there was marked infiltration in the interstitial area of seminiferous tubules when rabbits exposed to Sodium-Fluoride. No mature spermatozoa were seen in the lumen of the seminiferous tubules. Marked improvement near to the normal control group was seen when combined vit. D and E were given along with sodium fluoride for 30 days.

Thus it is concluded that our results indicate that sodium fluoride has a negative influence on liver and testis histological parameters in albino mice. In combination of vit. C it was contributed to the support of spermatogenesis to some extent with the recovery of testicular tissue.
LEGEND FOR FIGURES

PLATE – 5.1

Fig. A: Microphotographs of control liver of mouse showing hepatocytes (HC) with centrally placed nucleus (N), Cytoplasm in hepatocyte (Cy) – H & E. 100X

Fig. B: Microphotograph of control mouse liver at higher magnification showing hepatocytes (HC), Nucleus (N) and Kuffer cells (KC) - H&E. 450X
LEGEND FOR FIGURES

PLATE – 5.2

Fig. C:  Microphotograph of mouse liver under 7 days of sodium fluoride showing Necrotic changes in hepatocytes (NHC) - H&E. 100 X

Fig. D:  Micrograph of mouse liver under 7 days of sodium fluoride showing Moderate necrotic changes in hepatocytes (MNHC), Cytoplasmic degenerative changes in hepatocytes (NHC) – H&E. 450X
LEGEND FOR FIGURES

PLATE – 5.3

Fig. E: Microphotograph of mouse liver under 7 days of sodium fluoride + Vit. C showing Moderate degenerative changes in hepatocytes (MDGHC) – H & E. 100 X

Fig. F: Photomicrograph of mouse liver under 7 days of sodium fluoride + Vit. C showing Cytoplasmic degenerative changes in hepatocytes (CyDG) - H&E. 450X
LEGEND FOR FIGURES

PLATE – 5.4

Fig. G: Microphotograph of mouse liver under 30 days of sodium fluoride showing Necrosis in hepatocytes (NHC), Cytoplasmic degeneration (CyDG) pushing of nucleus to periphery of hepatocytes (PNP), Binucleated condition (BN) – H&E. 100 X

Fig. H: Microphotograph of mouse liver under 30 days of sodium fluoride showing pushing of nucleus to periphery of hepatocytes (PNP), Degeneration of Nucleus & cytoplasm in hepatocytes (DGNHCy), Binucleated condition (BN), Cytoplasmic degeneration (CyDG) – H&E. 450 X
Plate – 5.4

Fig. G

Fig. H
LEGEND FOR FIGURES

PLATE – 5.5

Fig. J: Microphotograph of mouse liver under 30 days of sodium fluoride+Vit. C showing Moderate degenerative changes in hepatocytes (MDGH) – H&E. 100 X

Fig. K: Microphotograph of mouse liver under 30 days of sodium fluoride + Vit. C showing Cytoplasmic Degeneration in hepatocytes (CyDG), Necrotic changes in hepatocytes (NHC), Binucleated condition – H&E. 450 X
Plate – 5.5
LEGEND FOR FIGURES

PLATE – 5.6

Fig. L: Microphotograph of control mouse testis showing seminiferous tubules (ST), Mature spermatozoa (MSP) Lumen of seminiferous tubule (LST) - H&E. 100 X

Fig. M: Microphotograph of control mouse testis at higher magnification showing Mature spermatozoa (MSP), Immature spermatozoa Spermatids (SP) – H&E. 450 X
Plate – 5.6
LEGEND FOR FIGURES

PLATE – 5.7

Fig. N: Microphotograph of mouse testis under 7 days of sodium fluoride showing moderate degenerative changes in seminiferous tubule (MDGST), Reduced spermatozoa (RSP) - H&E. 100 X

Fig. O: Microphotograph of mouse testis under 7 days of sodium fluoride at higher magnification showing moderate degenerative changes in seminiferous tubule (MDGST), Reduced spermatozoa in lumen of seminiferous tubule (RSP) – H&E. 450 X
Plate – 5.7
LEGEND FOR FIGURES

PLATE – 5.8

Fig. P: Microphotograph of mouse testis under 7 days of sodium fluoride + Vit. C showing Lumen of seminiferous tubule (LST), Moderate degenerative changes in seminiferous tubule (MDGST), – H&E. 100 X

Fig. Q: Microphotograph of mouse testis under 7 days of sodium fluoride + Vit. C at higher magnification showing Moderate degenerative changes in spermatids (MDGSPT), – H&E. 450 X
Plate – 5.8
LEGEND FOR FIGURES

PLATE – 5.9

Fig. R:  Microphotograph of mouse testis under 30 days of sodium fluoride showing severe degenerative changes seminiferous tubule (SDGST), Necrotic changes in spermatids – H&E. 100 X

Fig. S&T:  Microphotograph of mouse testis under 30 days of sodium fluoride showing necrotic changes in spermatids (NSPT) – H&E. 450X
Plate – 5.9
LEGEND FOR FIGURES

PLATE – 5.10

Fig. U: Microphotograph of mouse testis under 30 days sodium fluoride+Vit. C showing moderate degenerative changes in spermatids (MDGSPT) – H&E. 450 X

Fig. V: Microphotograph of mouse testis under 30 days sodium fluoride+Vit. C at lower magnification showing moderate degenerative changes in seminiferous tubules (MDGST) – H&E. 100 X
Plate – 5.10