Chapter-III

Histopathology
INTRODUCTION

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 investigation were carried out in the mid nineteenth century. Exposure of animals to contaminated water also causes severe pathological changes at the tissues level. Histology is a structural science and serves to complement the knowledge gained from the anatomy, physiology and pathology and it gives insight into the functioning of tissues and organs (Madhava Rao et al., 2009). 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, the study of microanatomy of specific tissues, 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 (Madhava Rao et al., 2009). 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 and Sted man’s medical dictionaries, 2005).

Histology and cytology are concerned primarily with morphologic 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 toxicants and pointed out the architectural damage to brain, gill, liver, kidney, heart, lung, muscle, testis, intestine in various animals (Shukla et al., 2001, Glynn, 2003; Garg et al., 2004; Sivaiah, 2006; Jayasankar, 2007; Madhava Rao, 2007; Nagarjuna, 2007 Savithri, 2009; Hari, 2010 and Sheerisha 2012).

Histology is the best and most direct method of studying xenobiotics by means of in vivo scanning and photographic presentation. 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. 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). 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.

It can be suggested that both morphological and biochemical assay should be applied for more accurate evaluation of pathological concepts. Moreover 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 systems of an organism. Toxicological histopathology gives useful data concerning the changes induced by chemicals at the tissue and cellular level. 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 prove to the external manifestations of the deleterious effects of chemicals (Jayantha Rao, 1982).

The study of abnormal cells and tissues is histopathology (Aughey and Frye, 2001). 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.

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. It is obvious that any chemical indult could cause pathological damage or injury to cells in an animal if it’s taken beyond the safe permissible limits. Susceptibility to chemical injury varies greatly among the tissues and cells of the same animal and more so among the tissues and cells of the same animal and in different animal groups. Different group of toxins that do not cause deterious effects of the portal or entry but they systematically affect, the tissue in which they get accumulated. Thus various chemicals with their varied mode of action affect different tissues here by bringing about certain architectural changes ultimately culminating in either death of the organism or making the organism less liable for its survival (Cavas and Ergent Gozukara, 2003).

Physiological studies alone do not satisfy the complete understanding of pathological conditions of tissues under toxic stress. Hence it is useful to analyze the histological aspects. Also, the severity of damage depends on the toxic potentiality of a particular compound accumulated in the tissue (Soni and Bhatnagar, 2005; Tisch et al., 2005). Several workers reported on the pesticides and pointed out the architectural damage to brain, gill, liver, kidney, heart, lung, muscle, testis, intestine in various animals (Pondy et al., 1997a; Shukla et al., 2001, Glynn, 2003; Garg et al., 2004; Madhveelatha, 2006; Sivaiah, 2006; Rajendra Prasad, 2007; Sukanya, 2007; Kishandar, 2007; Madhava Rao, 2007; Venkatchadrudu M, K. Radhakrishnaih, 2008; Kota Sobha et al., 2008; Subaredddy et al., 2009; Appa Rao et al., 2009; Hamadi Fetoui et al., 2009; Madhava Rao et al., 2009; Hooser and Earnes, 2010; Thomas Barks, 2010; Sands and T W Verlande, 2010).

In terrestrial and aquatic animals fertilizers, chemicals, and insecticides produce toxic effects in different tissues. For understanding the pathological conditions of animal, histological studies pave way to have a clear understanding as to how these chemicals can be injurious to the tissues. It is essential to have an insight into the histological analysis of the tissues. Some chemicals are toxic at even very low concentrations and these chemicals
necessarily impair the metabolic strategy of the animal physiologically. Compounds which enter the body via intestinal lymphatic system after oral feedings bypass the liver accordingly. The adrenal gland and testicular interaction appear to be depending upon overlapping function of the steroid hormone.

Histology gives the insight into the functioning of tissues and organs. In a precise sense, it is the study of cytoarchitectural change of the body which envisages the anatomy. Thus histology is a structural science and serves to complement the knowledge gained from the anatomy, physiology and pathology. If any chemical is taken beyond the safe permissible limits, it could cause pathological damage or injury to cells and in animal. Susceptibility to chemical injury varies greatly among the tissues and cells of the same animal and more so among different animal groups. The extent of severity of tissue damage is a function of the concentration and potentiality of toxic compound accumulated in the tissues (Jayanth Rao, 1982). Histopathological changes of different animals by metals have been reported by several workers (Akilendar Naidu, 1982 and Usha Rani, 1986).

Toxicants which are ubiquitous in nature have become integral part in the tissues of animals. Toxicants find their way into places far from application and accumulate in significant concentrations in the tissues of animals. Generally chemicals metals and pesticides accumulate to a greater extent in the liver (Edwards, 1973) which is the center for toxicant metabolism. Toxicants residues in the tissue cause serious physiological alterations even at low levels (Dikshith et al., 1974; Mathur et al., 1981). Johnson (1976) and Verma et al. (1979) pointed out that a prolonged period of exposure to chemical compounds, with very low concentration, results in the accumulation of more in the organs.

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.

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 adverse
effect 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.

Susceptibility to chemical injury varies greatly among the tissues and cells of the
same animal. 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). 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. Some other toxic compounds do not cause damage at the portal of entry but affects
the organs systematically in which they are accumulated.

Since the adrenal hormone has a direct influence on testicular function, the present
study has been aimed to understand the relationship between testicular function and
ammonia stress. The sperm production, motility and potentiality directly relate the function
of testis.

NORMAL HISTOLOGY OF RAT TESTIS:

The testis consists of two important functional components: the interstitial cells and
the seminiferous tubules. The seminiferous tubules give rise to spermatozoa through a
process known as spermatogenesis. The control of spermatogenesis is quite complex and
requires testosterone from the Leydig cells situated within the interstitium and a supportive
role by sertoli cells within the seminiferous tubule. Over the past decade, there has been
considerable scientific, regulatory and public concern for the potential role of stress induced
by environmental factors and their effect on male reproductive capacity. Studies have shown
that one in six couples of reproductive age present with infertility (Hull et al., 1995).
Different etiologies have been identified as a cause of male infertility (Pardon et al. 1997), Wang et al. 1997, Carbone et al. 1998, Hendin et al. 1999) and recent advances in the understanding of male fertility has implicated oxidative stress as the major causative factor (Aitken and Krausz 2001). Reactive oxygen species have been shown to damage almost all macromolecules of the cell, including membrane-bound polyunsaturated fatty acids, thereby impairing cellular functions (Lenzi et al., 2001).

Historical observations of the testis of the rat revealed that the seminiferous tubules contain all stages of spermatogonia and interstitial cells. The different stages of spermatogenesis are spermatogonia attached on the basement membrane of seminiferous tubule; and towards the lumen the primary spermatocytes, secondary spermatocytes and spermatids were found in lumen of the seminiferous tubules filled with sperms. Interstitial tissue showed clusters of Leydig cells.

The testis of humans and other mammals are highly susceptible to damage caused by genetic disorders, environmental or occupational exposure to chemicals or by other means. Several causes of testicular damage have been catalogued (Nebbia 1987, Ronis and Badgar 1996).

The testis surrounded by double layer of mesothelium i.e., inner tunica vaginalis is covered by outer tunica albuginea. The capsule is reflected into the median plane of the testis to form a partition “the mediastium” and gives off loose vascular connective tissue “the septula testis” to divide the testis into lobules to support the seminiferous lobules. The coiled seminiferous tubules are lined with a multilayered seminiferous epithelium of spermatogenic cells and sustentacular (sertoli) cells. They rest on a basement membrane and are surrounded by a lamellated connective tissue with myoid elements. The specific interstitial (Leydig) cells are found in the loose vascular connective tissue separating the tubule.

In the pre pubertal male, there are two cell types, the sustentacular cell and the spermatogonium, the immature male germ cell. The sustentacular cells are tall columnar extending from the basement membrane to the lumen of the tubule with a pale vesicular
basal nucleus and a prominent nucleolus. As the name suggests, the sustentacular cells support the later stages in the development of spermatozoa.

Spermatogonia lie next to the basement membrane and are small round cells with a dark staining nucleus. The primary spermatocyte divides meiotically to form two secondary spermatocytes which divide immediately to form two haploid spermatids. These are small cells with a spherical nucleus and lie close to the lumen of the tubule. The spermatids move into recesses in the sustentacular cells and metamorphose into spermatozoa, shedding the excess cytoplasm into the lumen of the tubule (Aughey and Fryc, 2001).

Histometric studies on the testis proved the strong evidence to see the difference between control and experimental tissues. A positive relationship exists between the tubular diameter and the speratogenic activity of the testis.

**Results:**

Histological observations of the testis of the control rat consist of seminiferous tubules, spermatogenesis and inter-tubular elements. The semineferous tubules shows normal spermatogenesis with all cell types and well developed interstitial cells. Each seminiferous tubular wall with the outermost basement membrane resting on the basement membrane is the spermatogonia and the sertoli cells. Towards the lumen, the primary spermatocytes, secondary spermatocytes and spermatids adhere to the sertoli cells. Sperms are seen with heads embedded in the sertoli cells the tails lying in the lumen (fig.1; Group-I).

To know the effect of ammonia stress on the histological alternations in the testis of adult rats, transverse section of testis exposed to ammonia sulphate has been taken. The histological studies of testis in ammonia sulphate treated rat testis showed decreased number of spermatocyte, spermatids and sperms in the lumen of semineferous tubules and mild clumping of spermatozoa in seminiferous tubules and interstitial tissues contain cluster of leydig cells. Pronounced pathological changes such as severe degenerative changes in spermatozoa and epithelial layer of seminiferous tubules besides light necrotic changes in the interstitial cells loss of connective tissue and degenerative Leydig cells, disturbed lumen and accumulation of oedematous fluid in the interstitium, were observed the semineferous
tubules are disorganized. The germinal epithelium, spermatogonia, spermatocytes and spermatids are severely damaged and degenerated. Leydig cells are highly compressed on one side to disruption on the other side. The degeneration was observed where in the tubules show necropsied spermatogenic cells and the lumen was empty of active sperms. (Fig -2; Group- II).

The structure of seminiferous tubules was observed in both control and ammonia treated rats. The control rat testis showed the clear lumen with all spermatogonial elements under enlarged condition. The ammonia treated rat testis showed the cell debris in the oedematous fluid in the lumen of the seminiferous tubule with cessation of spermatogenesis, necrotic spermatogonia, necrotic spermatids and a few residual spermatozoa along with the atrophied Leydig cells when compared to control rats. The photographs showed the connective tissue along with the seminiferous tubule of both control and ammonia treated rats. In the testis of control rat the normal germinal epithelium, leydig cells and sertoli cells were observed under enlarged condition. In ammonia treated rat, testis showed degenerative epithelium, atrophied leydig cells and sertoli cells when compared to the control rats.

Ammonia sulphate with vitamin-c treated rat testis showed recovery changes when compared with experimental animals in the present investigation. All these degenerative changes in spermatids, clumped spermatozoa, increased area of lumen of seminiferous tubules, degeneration in leydig cells, degenerative in connective tissues, structural degenerative changes and atrophy of seminiferous tubules and fragmentation of seminiferous tubules are recovered in ammonia sulphate with vitamin-.C group. Fig .3; group III).
TESTIS:
CT = Connective Tissue
DLC = Degenerative Leydig Cells
LCT = Loss of Connective Tissue
LST = Lumen of Seminiferous Tubules
SD = Spermatids
SDC = Structural Degenerative Changes
SF = Sperm Flagella
SG = Spermatogonia
ST = Seminiferous Tubules

Group-I:
- The normal cyto-architecture of the testis with lower magnification (100X); And Higher magnification (400X).

Group-II:
- The distraction of testis shows loss of connective tissue and leydig cells indicates degenerative changes with lower magnification (100X); And Higher magnification (400X).

Group-III:
- Regenerative changes in testis takes place and shows similar to normal tissue with lower magnification (100X); And Higher magnification (400X).
LEGEND FOR FIGURES

PLATE – 1.

Fig. 1.1: Transverse section of control rat testis showing seminiferous tubules (SFT) with spermatids and mature spermatozoa with a outer membrane, theca albuginea (TA) besides leydig cells (LC) in between seminiferous tubules - H&E. 100 X

Fig. 1.2: Transverse section of control rat testis at higher magnification showing spermatids (SPD), spermatozoa (SP) and theca albuginea (TA) – H&E. 400 X

SF = Sperm Flagella
SD = Spermatids
SG = Spermatogonia
Transverse section of control rat testes showing seminiferous tubules (SFT) with spermatids and mature spermatozoa with an outer membrane, theca albuginea (TA) besides Leydig cells (LC) in between seminiferous tubules - H&E. 100 X & 400X.
LEGEND FOR FIGURES

PLATE –2

**Fig. 2.1:** Transverse section of rat testis under ammonium sulphate showing loss of connective tissues (LCT); Degenerative Leyding Cells (DLC); sturcutral Degeneratives (SDC) degenerative changes in seminiferous tubules (DGSFT) h&e. 100 X

**Fig. 2.2:** Transverse section of rat testis under ammonium sulphate at higher magnification showing loss of connective tissues (LCT); Degenerative Leyding Cells (DLC); sturcutral Degeneratives (SDC) degenerative changes in seminiferous tubules (DGSFT) h&e. 400 X.

$LCT = \text{Loss of Connective Tissue}$

$DLC = \text{Degenerative Leydig Cells}$

$SDC = \text{Structural Degenerative Changes}$
Transverse section of rat testes under ammonia sulphate showing loss of connective tissues (LCT); Degenerative Leyding Cells (DLC); sturcutral Degeneratives (SDC) degenerative changes in seminiferous tubules (DGSFT) h&e. 100X & 400X.
LEGEND FOR FIGURES

PLATE – 3

Fig. 3.1: Transverse section of rat testis under ammonium sulphate and vitamin-c showing seminiferous tubules (ST) with spermatids and mature spermatozoa with an outer membrane, connective tissue (CT) - H&E. 100 X

Fig. 3.2: Transverse section of rat testis under ammonium sulphate and vitamin-c at higher magnification showing seminiferous tubules (ST) with spermatids and mature spermatozoa with an outer membrane, connective tissue (CT)- H&E. 100 X

CT = Connective Tissue
ST = Seminiferous Tubules
SG = Spermatogonia
Transverse section of rat testes treated with ammonia sulphate and vitamin-c showing seminiferous tubules (ST) with spermatids and mature spermatozoa with an outer membrane, connective tissue (CT).

- H&E. 100 X &400X.
Discussion:

The androgens are responsible for the maintenance of the normal growth and spermatogenic function (Petu et al., 1988). The androgen insufficiency may likely to take place on adrenalectomy leading to alterations in the spermatogenesis. The reduced weight and testis weight on ammonia treated in the present study might also be responsible for the reduced sperm count. Several reasons can be attributed to the depleted sperm count level on ammonia stress. The reproductive functions are affected by stress conditions (River and Rivest, 1991). A stress response involves the activation of neural and hormonal networks which lead to an increase of catecholamine and glucocorticoid section. The catecholamine augments testosterone plasma level by elevating blood flow into the testis and they regulate the testosterone in blood (Sapolsky, 1986). Hence, the decreased catecholamine production might be responsible for the reduced sperm count. The observed histopathological changes in testis on ammonia stress in the present study can be attributed to the above reason for the sperm count.

Nadia Ait Hamadouche et al., 2013 reported the normal architecture of testicular seminiferous tubules and interstitial spaces in the control rats. Rats that were treated with lead acetate showed complete necrosis and sloughing of all layers of seminiferous tubules. The testes of rats treated with vitamin E showed mild to moderate edema, congestion with minute foci necrosis and hemorrhage and the protective effect of vitamin E against testicular damage induced by lead acetate toxicity in experimental animals. In the present study, we evaluated the protective role of vitamin-c against testicular damage induced ammonia sulphate toxicity treated testis.

Histology of testis showed significant degenerative changes in the structural organization of testis on ammonia treated rats when compared to control rats. The diameter of the seminiferous tubules was significantly reduced in testis on ammonia treated, suggesting direct impact of adrenal hormone insufficiency on the structural integrity of the testis. Since, the seminiferous tubules were associated with regulatory mechanism that control the flow of elements across the blood-testis barrier and provide a structural integrity for the development of the sperm in testis, the observed degenerative changes like decreased seminiferous tubule diameters on ammonia influence the direct effect of adrenal hormone.
insufficiency on structural integrity of the testis. Several abnormalities were observed in the spermatozoa of the testis, such as loose contact of the mitochondrial helix with plasma membrane, loss of mitochondria, retention of cytoplasmic droplet, fracturing of outer dense fibers and presence of both the mid piece and the principal piece cross sections in a common plasma membrane. The predominant defect was the frequent presence of equidistant, cross, sectioned mid pieces of tail embedded in a common cytoplasm. The defects are indicative of loss of sperm motality (Shalini and Bausal, 2007).

Y.V. Kishore reddy et al., 2010 reported that testosterone mediated partial recovery of testicular damage induced by carboplatin induced toxicity in rat. Similarly vitamin-c has partial recovery on testicular damage on testis.

Germinal epithelium constitutes sertoli cells or spermatogenic cells out of which the sertoli cells provide nutrients for the growth and development of spermatogenesis (Majumdar, 1980). Marked changes in structuraly organization of the both the cells were observed on ammonia treated rats. In view of the deranged structural organization of germinal epithelium on ammonia treated rats, the possible impact of adrenal hormone insufficiency in the process of spermatogenesis can be expected. Ammonia also showed significant impact on the interstitium which was occupied with oedematous fluid formed by cellular death of germinal epithelium on ammonia treated rats.

Thus, it is concluded that ammonia showed profound influence in the testicular structural organization leading to degeneration of germinal epithelium, decreased diameter of seminiferous tubules and atrophy of Leydig cells. Moreover, it is evident that definite correlation exists between effect of ammonia and testicular function.

Ayinde et al., 2012 reported that oral administration of lead was responsible for histological damage and disturbances in the metabolism of male reproductive organs, reduction in the seminiferous epithelium accompanied with a reduction in the lumina spermatozoa. Nonetheless ameliorative effects where observed with vitamin C and Vitamin E treatment. Vitamin C proved to be ameliorative by improving replenishing testes reducing testicular histology towards normal physiology in our present study.

When ammonium sulphate was injected to the rats, the histological examination showed different pathological lesions in testis. Reports of infertility among pesticide workers
are increasing (Whorton et al., 1997). Cypermethrin is known to reduce fertility in male rats through affecting testosterone, follicle-stimulating hormone and luteinizing hormone and the number of cell layers of the seminiferous tubules as well as to cause congestion and hemorrhages in testes (Elbetieha et al., 2001). Khan et al., (2003) observed the loss of spermatozooids, and complete dearrangement of cellular organization of testis in rats treated with novel phosphorothionate.

The damage to the sperm producing seminiferous tubules has been reported after exposure to the pesticide and they greatly reduced in diameter (Omura et al., 1995). Pratap Sing and Vandana Sing, (2008) observed condensation of spermatogenic cells, vacuolization in tubular epithelium and disruption in Leydig cells in Heteropneustes fossilis treated with cypermethrin. Manna et al. (2004) reported edema between seminiferous tubules, vacuolation and hyalinization in the tubules of rats testes exposed to α-cypermethrin.

Gopala Reddy et al., 2013 reported that histological, sections of testis in rats’ revealed vacuolation, degeneration of seminiferous tubules and further germinal cells were detached from basement membrane, disrupted basement membrane, increased interstitial spaces, presence of very few Leydig cells, severe congestion in interstitial spaces and tunica albuginea was observed in imidacloprid over 4 weeks on histological and ultra structural alterations in testis in experimental rat.

Sivaiah, 2006 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.

Vitamin-C proved to be ameliorative by improving replenishing testes reducing testicular histology towards normal physiology in our present study. Similar changes as reported by earlier studies of Omura et al., 1995, Gopala Reddy et al., 2013, were observed in the present study in the experimental rat administrated with ammonia sulphate. These changes seems to decline and degeneration has not probably taken place in rats administrated with vitamin-c along with ammonia sulphate.