HISTOLOGY
INTRODUCTION

The architectural dynamics of a tissue is very essential for maintaining the tissue integrity and for effective physiological, biochemical and metabolic functions. The cellular and subcellular 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. 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 investigations carried out in the nineteenth century (Virchow, 1858). 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).

Testis is a vital organ in male reproductive system. Each testis is an egg shaped body hanging from the spermatic cord into the cavity of the scrotum. Two membranes, tunica vaginalis and the tunica albuginea, cover the testis. The visceral layer is a continuation of the parietal layer of the tunica vaginalis of the scrotum. In between the parietal and visceral layers there is a narrow space filled with a fluid, which facilitates the movement of the testis within the scrotum. Inner to the visceral layer is a thin membrane of collagenous connective tissue, which is called the tunica albuginea. It covers the entire testis. On the posterior side of the testis this connective tissue accumulates in mass called mediastinum testis. Inner to tunica albuginea there is aerolar
connective tissue with plenty of blood vessels called tunica vasculosa. Both tunica albuginea and tunica vasculosa give out partitions into the testis containing 200 to 300 conical compartments that form as many lobules of the testis.

The germinal epithelium is a multilayered structure formed by two kinds of cells, sertoli cells and germ or spermatogenic cells. Sertoli cells are bigger, somewhat triangular cells with broad bases and indistinct outlines. These cells are fewer in number and each one has a big nucleus. The fully formed sperms remain attached to the edges of the sertoli cells. Spermatogenic cells are several kinds with 4 to 8 layered in the epithelium between the basement membrane and the lumen. Four kinds of spermatogenic cells can be distinguished in the wall of tubule. They are a) spermatogonia, b) primary spermatocytes, c) secondary spermatocytes and d) spermatids. In rat, spermatogenic cells at similar stages of development occupy the same levels in the germinal epithelium of a seminiferous tubule. Therefore in a section they look like concentric rings of similar cells (Majumdar, 1980).

A few reports stated that the sperm count significantly lowered on adrenalectomy (Devendra Naidu, 2000) and also the structural alterations of sperms were known to coexist with infertility (William Bloom et al., 1962). In adrenalectomized rats due to decreased zinc concentration, the leydig cells are atrophied, germinal epithelium was degenerated and the seminiferous tubules were full of oedematous fluid (Nair et al., 1995). But increased zinc
concentration (along with degeneration and oedema) has been reported from testis (Nair et al., 1987), liver (Nair et al., 1988), intestine (Nair et al., 1989) of adrenalectomized rats. Administration of di (2 ethyl hexyl) phthalate (DEHP) into rat brain revealed moderate focal degenerative changes in the cerebrum and cerebellum and neurons had become shrunken, eosinophilic and contained pyknotic nuclei (Dhanya et al., 2004).

Epididymis consists of three parts. They are 1) the caput epididymis: which lies at the end of the testis to which the spermatic cord is attached, 2) the corpus epididymis or central body and 3) the cauda epididymis which is present at the opposite pole of the testis. Holocrine and principal cells are present in the rat epididymis. The lining epithelium of the epididymis is composed of two types of cells, narrow and tall columnar cells and round basal cells. The columnar cells bear numerous very long microvilli, which are largely nonmotile and named as stereocilia. The basal cells, which are numerous in number, rest on the basement membrane. They are spherical in shape and with almost central round nuclei. These cells are thought to be the progenitors of the columnar cells. Robaire and Hermo (1988) have revealed that the histoarchitecture of the various cell types in the ductus epididymal epithelium of the control rats. In phosphamidon treated rats, the cytoplasm of epididymis vacuolrized and nucleus was displaced towards the apical end, with the decrease in cell height and width. The cytoplasmic granules were increased in size and also a depletion of circulating levels of androgens in the principal
cells of the caput epithelium of rats (Akbarsha and Sivaswamy, 1998). Hence, it implies that the caput-corpus epididymis has a greater androgen requirement than that the cauda epididymis (Robaire and Hermo, 1988) and is most sensitive to androgen deprivation (Robaire et al., 1977).

The penis consists of three cylindrical masses of prominent erectile tissues, two dorsolateral corporacavernosa and one ventral corpus spongiosum. Each of them is encapsulated by a dense capsule tunica albuginea, which is composed of collagenous and elastic fibres. All these three erectile tissues and their capsules are ensheathed by a common areolar connective tissue and elastic fibres. A thin skin finally covers the penis externally. During sexual stimulation the subdivisions of cavernosa tissue become filled with blood to the limit permitted by encasing connective and the organ becomes erect.

In rats ovary lies with in ovarian bursa. A layer of epithelium lines each ovary externally, which is continuous with mesothelial cells. They are flattened but epithelial cells are of cuboidal or columnar shape. Ovary has two distinct zones a central deeper portion, the medulla or zona vasculosa and a broad outer layer, the cortex. The medulla includes clusters of stromal cells and loose connective tissue rich in elastic fibres, blood vessels, lymphatic and nerves. The cortex includes a supporting cellular stroma and a number of follicular structures, ovarian follicles. The stromal cells are spindle shaped with elongated nuclei. The cortical stroma underneath the surface epithelium
forms a denser fibrous layer, the tunica albuginea composed of a few cells scattered in the closely packed collagenous fibres.

The uterus has three morphologically distinct regions, dome-shaped fundus, major swollen body (corpus) and a narrow lower cylindrical cervix. The corpus consists of an outer thin serosa (perimetrium), which is made up of a layer of flat, polygonal squamous cells, a middle thick muscularis (myometrium) and inner mucosa (endometrium). During pregnancy, the sex hormone possibly causes hypertrophy (increase in size) and hyperplasia (increase in number) of the muscle fibres. During the post-delivery stage, the muscle fibres retain their normal size and number. The endometrium is lined by an epithelium with a layer of columnar cells, some of which may be ciliated. The thickness of the endometrium varies dramatically through out the menstrual cycle in response to ovarian hormones. The uterus was interdependent of ovarian hormones. The uterine luminal epithelial cell height increases with estrogen treatment, while the uterine tissues atrophies after ovariectomy and progesterone treatment of rats (Oner et al., 2002). Suppression of the corpus luteum volume has been reported in adrenalectomized rats (Arora et al., 1994).

The vaginal wall consists of three coats mucous, muscularis and fibrosa. The mucous membrane is a stratified squamous epithelium, muscularis has longitudinal smooth muscle fibres, which are continuous with the myometrium of the uterus, and the fibrosa is composed of dense fibrocollagenous tissue
containing numerous thick elastic fibres, which binds the vagina with the surrounding structures (Verma, 2001). Martin and Claringbold (1960) and Kapshikar (1979) were reported changes in vaginal histology during estrus cycle. At mid cycle estrogen stimulation maximizes epithelial thickness, glycogen accumulation and decrease in pH of vaginal fluid (Knobil and Neill, 1998). Rats treated with sildenafil showed the increase of fibrocollaginous tissue, the enlargement of capillaries and the increase of connecting tissue elements in the corpus cavernosum of female reproductive tissues (Kilinic et al., 2003).

A significant loss of the weight of ovary and uterus, consequently high accumulation of arsenic in the plasma, ovarian follicular and uterine cell degeneration characterized by a high number of regressing follicles and a reduction in the uterine luminal diameter were reported in sodium arsenite treated rats (Chattopadhyay Sandip et al., 2003). In estrogen (E₂) treated rats, uterus showed increased cell proliferation at 24h. Thus, the thickening of the endometrium seen after adrenomedullin (ADM) was due to hyperplasia and edema rather than cell division (Ikeda et al., 2004).

The liver is the largest gland of the body and located upper and right part of the abdominal cavity immediately below the diaphragm. It is ensheathed by a delicate capsule of Glisson made up of connective tissue. There is a delicate layer of tunica serosa around the capsule. The components of the liver include hepatocytes, hepatic venule, portal veins and sinusoids.
Hepatocytes are polyhedral cells having central spherical nuclei with one or more prominent nucleoli. Sinusoid is limited on either side by plates of hepatocytes. A thin discontinuous highly fenestrated endothelium and scattered phagocytic stellate cells, kuppfer cells line the sinusoids. These cells have a large vascular nucleus and prominent branching pseudopodial processes. Shukla et al., (2000) reported that in carbon tetra chloride (CCL₄) treated rats the liver exhibited an extensive degenerative leison, vacuolization of hepatocytes and fatty degeneration.

Thus the above literature revealed structural changes in the hepatic and reproductive tissues of animals exposed to stress. As this line of information provides support for the biochemical alterations, haematological changes and level of hormonal imbalance, in the present study light microscope observations are made in the male and female reproductive tissues and liver of both ADX male and female albino rats.

RESULTS:

Under this study, the transverse sections of the liver, epididymis, penis and testis in male adrenalectomized (ADX) rats and liver, uterus, vagina and ovary in female ADX rats at day 15 and day 30 of experimentation, besides those of male and female shamoperated (SO) rats were photographed and are presented for observation in plates I to VIII.

The normal histoarchitecture of male liver included the hepatocytes, portal veins, sinusoids and kuffer cells along with blood cells in the central
vacuole. Hepatocytes were polyhedral cells having centrally located mono or binuclei with distinct nucleolus. Many cells were binucleated. Hepatocytes exchanged substances with sinusoids, bile canaliculi and the kuffer cells (plate-Ia). The liver in 15 days of ADX male rats showed a mild degree of cellular damage, which included destruction of hepatocytes with darkened nuclei. In few regions sinusoidal spaces were wide while in another regions hepatocytes were arranged in compact manner (plate-Ib). The photomicrograph of plate-Ic was 30 days of ADX male rat liver, which revealed severe cytoplasmic and nuclear damage, sinusoidal spaces were wider than at day 15 of ADX male rat liver. More number of kuffer cells was present in central vacuole.

The histoarchitecture of the epididymis of SO male rats included a number of tubules each consisted of epithelial membrane associated with rounded principal cells and columnar cells. In the lumen of each tubule a dense fluid was present with a number of spermatozoa (plate-IIa). Plate-IIb is the photomicrograph of epididymis at day 15 of male ADX rats. It exhibited a decreased tubular diameter and lumen diameter. The epithelial membrane was disrupted here and there and the tubular fluid became less concentrated with less number of spermatozoa. The space between the two tubules considerably increased. Severe damage in epithelial membrane of cauda epididymis with maximum degeneration in principal and columnar cells was observed at day 30
of male ADX rats. The tubular fluid disintegrated to a greater extent with a fewer number of spermatozoa (plate-IIc).

The normal histology of penis consisted of three cylindrical masses of prominent erectile tissues, two dorsolateral corpora cavernosa and one ventral corpus spongiosum. Each of them was encapsulated by a dense capsule, tunica albuginea, which was composed of collageneous and elastic fibres. Plate-IIIa showed all the three erectile tissues, and each of these tissues composed of a network of many large endothelium lined interconnecting venous spaces or lacunae. The spaces were surrounded by connective tissue and smooth muscle fibres. A mild damage was seen in areolar connective tissue and elastic fibres of penis of 15 days ADX rats with widen of interconnecting spaces (plate-IIIb). The photomicrograph of plate-IIIc showed that at 30 days the three erectile tissues of penis overlapped with irregular arrangement with the congestion of areolar tissue. The tissue cells appeared pyknotic and haemorrhagic spots were seen at few regions.

The shamoperated rats of each testis contained many seminiferous tubules. It had a central lumen lined by a specialized stratified epithelium. Most of its cells were spermatogonium, spermatid and spermatozoan while some of these were supporting sertoli cells. In between the seminiferous tubules, there were blood vessels and leydig cells (Plate-IVa). The seminiferous tubules of ADX rats on day 15 revealed the presence of oedematous fluid in the interestium, cessation of spermatogenesis with
necrotic spermatogonia and few karyolytic primary spermatocytes and few residual spermatozoa in the lumen and atrophy of leydig cells, while the seminiferous tubular diameter decreased over to SO rats (plate-IVb). On day 30, the degenerative changes were clearer wherein the seminiferous tubules exhibited oedematous fluid in the interstium and in the lumen of the tubules. Cell debris was also seen in the oedematous fluid of the lumen of the tubules. Along with the loss of the cellular identify of the germinal epithelium, spermatogenesis was completely arrested. Leydig cells were atrophied, while a seminiferous tubular space was with spermatozoa pushed from the tubular epithelial membrane. The hexagonal shape of the seminiferous tubule was lost and it appeared as an irregular spherical shaped (Plate-IVc).

On comparison with the normal histoarchitecture of males, in females the hepatic cells of the liver exhibited minor variations. The hepatic cords are in compact manner; most of the hepatocytes shown with different staining properties. Hyperplasia was also observed in some regions. Hepatocytes were mono or binucleated with distinct nucleoli (Plate-Va). On day 15, the liver of ADX female rats showed widening of sinusoidal spaces in few regions, while the central vein was filled with debris. Further, karyopyknotic nuclei were observed in perivascular region. A few haemorrhagic spots were also seen here and there (Plate-Vb). The photomicrograph of plate-Vc revealed that most of the nuclei of hepatocytes of the liver of female ADX rats at 30 days became fade and shrunken exhibiting vacuolization, while karyolysis was also
observed in few nuclei. The hepatic cells appeared necrotic with the loss of their integrity. Lots of haemorrhagic spots were clearly noticed with the presence of blood cells in the central veinules.

The normal histology of uterus wall consisted of an outer thin serosal (perimetrium) layer. It had polygonal squamous cells followed by a thin layer of areolar connective tissues and a middle thick muscularis (myometrium) in which bundle of smooth muscle fibres were held together by connective tissue. Inner mucosal (endometrium) layer was also seen intact in female SO rats (Plate-VIa). On day 15, a mild atrophy was noticed in the cells of all the layers and a marked thickening appeared in the endometrium (Plate-VIb). On day 30, thinning of myometrium with the dissolution of muscle fibres and increase in the degree of cellular degeneration in perimetrium and endometrium were observed. Clear-cut blood clots were seen in the uterine glands of endometrium, with the increase in vacuolization (plate-VIc).

The vaginal wall consisted of three coats, the mucous, muscularis and fibrosa. They were rich in polymorphonuclear leucocytes, lymphocytes (Plate-VIIa). On 15 days of ADX, the vagina showed less number of leucocytes and wide spaces appeared between elastic fibres of fibrosa. The squamosal cells of mucosal layer appeared pycnotic (Plate VIIb). At 30 days on ADX the vagina exhibited the presence of very less number of leucocytes in mucous, while the elastic fibres of muscularis mostly disappeared. Blood clots were also seen in fibrosal layer. The vaginal gap was also greatly reduced (Plate VIIc).
The female SO rat ovary had the normal pattern of follicular development. The photomicrograph of plate-VIIIa showed the presence of different types of follicles indicating the proper development of them during estrous cycles. The occurrence of primary, secondary, tertiary and graffian follicles indicated different stages of the development of oocytes. More than one layer of granulosa cells was present in the oocyte. The photomicrograph of plate-VIIIb indicated the follicular development in the 15 days of ADX rats ovary in which absence of all the stages of developing follicles was conspicuous. The nuclei became condensed and reduced in corpus luteum and blood clots were seen in the stromal cells of the follicle. On day 30 the ovary was seen with the breakage of the membrane of the oocytes and reduced number of granulosa cells. Vacuolization appeared in the oocytes. The different stages of the follicle were not seen (Plate-VIIIc).

DISCUSSION:

The morphological appearance of a tissue is documented evidence on the adverse effect of adrenalectomy in an animal. In the present study, compared to the SO rats, the progressive degenerative changes in the organs of liver, epididymis, penis and testis in male ADX rats and liver, uterus, vagina and ovary in female ADX rats provide support to the changes observed in protein levels.

The appearance of degenerative changes in the liver of male and female ADX rats at day 15 and at day 30 experimentation support the metabolic
disorders observed in it. A mild degree of cellular damage, which includes destruction of hepatocytes with darkened nuclei and widening of sinusoidal spaces in few regions at day 15 in the liver of ADX male rats; and severe cytoplasmic and nuclear damage, and still more wider sinusoidal spaces at day 30 suggest that adrenalectomy leads slow degeneration of the metabolic tissues like liver, thereby the protein synthetic ability of it decreases leading to the loss of its weight. It also leads to increase in ammonia production and impaired oxidative metabolism. The female ADX rats showed widening of sinusoidal spaces in few regions while the central vein is filled with debris. The nuclei are karyopyknotic in perivascular region at day 15; but greater degeneration is observed at day 30 where in the nuclei of hepatocytes shrunken, some of them exhibited more vacuolization, while karyolysis is also observed in few nuclei. These changes support that the female rats prone to more vulnerability on ADX stress than the male ADX rats; it is probably due to more protein degradation and increased oxidative stress. A similar sex differences in histopathological changes was observed under various stress conditions in the humans and rats (Elinder et al., 1976; Udita Gubrelay et al., 2004) that support the changes observed in the present investigation.

The histoarchitecture of the epididymis in male ADX rats exhibited decreased tubular diameter, and the disruption of epithelial membrane here and there and the tubular fluid became less concentrated with less number of spermatozoa at day 15. Severe damage in epithelial membrane of cauda
epididymis with maximum degeneration in principal and columnar cells is observed at day 30. These changes revealed the damage to the male reproductive structure due to lowered corticular steroids. The adrenalectomy might have resulted in the gradual atrophy of reproductive structure; consequently it decreased in sperm reserve in epididymis (Monteire et al., 1989) on ADX. Sarkar et al., (1997) reported that decrease in sperm reserve appears to be a reasonable cause for reduction in weight of epididymis in the rats exposed to drugs.

The seminiferous tubules of testis also revealed perceptive changes like concentration of oedematous fluid in the interstitium, cessation of spermatogenesis with necrotic spermatogonia and few karyolytic primary spermatocytes and atrophy of leydig cells while the seminiferous tubular diameter decreased at day 15. On day 30 the degenerative changes are clearer wherein the loss of the cellular identity of the germinal epithelium and complete arrest of spermatogenesis are noticed, while seminiferous tubular spaces is filled with spermatozoa pushed from the tubular epithelial membrane. These changes indicate perturbation in hormonal levels, increased activity of LDH and decreased in testicular weight. Nair et al., (1995) and Devendra Naidu (2000) also reported decreased sperm count and atrophy of leydig cells in the testis of rats on ADX. Cemil et al., (2002) reported similar pathological changes in testis of rats exposed to ELF (extremely low frequency) magnetic field.
A mild damage is seen in areolar connective tissue and elastic fibres of penis of rats at day 15 on ADX, with the widening of interconnecting spaces. On day 30, the three erectile tissues of penis overlapped with irregular arrangement and congestion of areolar tissue. The tissue cells appeared pyknotic and haemorrhagic spots are seen at few regions. These degenerative changes can be correlated to the decreased availability of glucocorticoids and cellular death (Nair et al., 1995). Such changes may appear due to the lower level of testosterone in circulation on ADX (Mann et al., 1982).

A mild atrophy is noticed in the cells of all the layers of uterus and a marked thickening appeared in endometrium of uterus of ADX rats at day 15. On day 30 the thinning of myometrium with the dissolution of muscle fibres and increase in the degree of cellular degeneration in perimetrium and endometrium are observed. Clear-cut blood clots are seen in the uterine glands of endometrium with the increase in vacuolization. These changes suggest lowering of estrogen levels and decreased uterine weight as the uterus undergoes drastic structural and functional changes in response to decreased estrogen level (Ikeda et al., 2004). In contrast, administration of estrogen rapidly increases the micro vascular permeability and increased weight of the uterus (Cullinan-Bove and Koos, 1993). On day 15 the vagina of ADX rat showed less number of leucocytes and wide space between fibres of fibrosa. At day 30 of ADX the vagina exhibited the presence of still less number of leucocytes in mucous, while the elastic fibres of muscularis mostly
disappeared and the vaginal gap is also greatly reduced. They indicate less cornification reactions due to decreased level of estrogen in the circulation. Butcher et al., (1974) reported that sex steroids especially influence on the cyclical changes in various stages of estrous cycle, lower levels of them results in less cornification reactions in the vagina of ADX rats. The ovary of ADX rats showed at day 15 the absence of all the stages of developing follicles, and blood clots are seen in the stromal cells of the follicle. On day 30 the ovary exhibited the breakage of the membrane of oocytes and reduced number of granulosa cells. It can be attributed to insufficient gonadotrophins and/or decreased ovarian activity or decreased weight in ovary. Arora et al., (1994) suggested that suppression of ovarian steroidogenesis by the reduction of Δ^5βHSD (hydroxyl steroid dehydrogenase) activity in ADX rats leads to structural degeneration of ovary.

On the whole, the adrenalectomy causes an irrecoverable damage to the reproductive organs of male and female rats. The structural disorganization is considerably worse in female ADX rats than the male ADX rats especially at day 30 of experimentation which indicates that the degree of impact of adrenalectomy is more in females which progressed with duration as seen in protein metabolism, haematological parameter and hormonal imbalance. Thus, the histopathological changes in rats on ADX are dependent on the sex of the animal and duration of the adrenalectomy.
PLATE – I

a: Transverse Section of liver of male sham operated (SO) rat.

b & c: Transverse Section of liver of male adrenalectomized (ADX) rats at day 15 (b) and at day 30 (c).
PLATE – I

a

b

c
PLATE – II

a: Transverse Section of *epididymis* of male sham operated (SO) rat.

b & c: Transverse Section of *epididymis* of male adrenalectomized (ADX) rats at day 15 (b) and at day 30 (c).
PLATE – III

a: Transverse Section of penis of male sham operated (SO) rat.

b & c: Transverse Section of penis of male adrenalectomized (ADX) rats at day 15 (b) and at day 30 (c).
PLATE – III

a

b c
PLATE – IV

a: Transverse Section of testis of male sham operated (SO) rat.

b & c: Transverse Section of testis of male adrenalectomized (ADX) rats at day 15 (b) and at day 30 (c).
PLATE – V

a: Transverse Section of liver of female sham operated (SO) rat.

b & c: Transverse Section of liver of female adrenalectomized (ADX) rats at day 15 (b) and at day 30 (c).
PLATE – VI

a: Transverse Section of *uterus* of male sham operated (SO) rat.

b & c: Transverse Section of *uterus* of male adrenalectomized (ADX) rats at day 15 (b) and at day 30 (c).
PLATE – VII

a: Transverse Section of *vagina* of female sham operated (SO) rat.

b & c: Transverse Section of *vagina* of female adrenalectomized (ADX) rats at day 15 (b) and at day 30 (c).
PLATE VII
PLATE – VIII

a: Transverse Section of \textit{ovary} of female sham operated (SO) rat.

b & c: Transverse Section of \textit{ovary} of female adrenalectomized (ADX) rats at day 15 (b) and at day 30 (c).
PLATE – VIII

a

b
c