RESULTS

The present study is conducted on 50 male adult albino rats. They were divided into two groups : Group (1) included (35),rats divided into thirty rats treated by flutamide prenatally and five rats control. Group (II) included (15) rats divided into ten rats treated by flutamide postnatally and five control rats.

Control groups:

Morphologically: both testes are present within the scrotal sac, they are ovoid in shape, and the weight of each testis is about 4.13 ± 0.09 gm (table 1 and fig.9 & 10). The penis appears with regular smooth glans penis, rounded prepuce and the external urethral opening present on the top of the glans penis (fig.7 & 27). Also the distance between the base of shaft of the penis and the anus (ano-genital distance) is about 3.67 ± 0.19 cm in length (table 3 and fig.7). After removal of the hair of the anterior abdominal wall the skin appears clear from the areola and the nipple (Fig.7). Then after dissection of the anterior abdominal wall the genital organs as the seminal vesicles are observed as highly convoluted gland present behind the urinary bladder (fig.9).

The prostate gland appears below the urinary bladder as two lobes like structure, also the genital ducts as the vas deferens (fig.9 & 61) and the epididymis . The average weight of the epididymis is 0.75 ± 0.08 gm (table 2), and is formed of head above the testis, body behind it and tail below it (Fig. 9 & 54).

Histologically: The microscopic structure of the adult control rat testes:

a- Light microscopic structure: The cross section of the adult control rat testis consists of transverse sections of the seminiferous tubules with interstitial tissue in between . The seminiferous tubules are lined by a stratified germinal epithelium and Sertoli cells. The germinal epithelium is arranged in raws and consists of spermatogonia, primary spermatocytes, spermatids and spermatozoa. The spermatogonia are founded on the basement membrane of the seminiferous tubule. Their nuclei are spherical or ellipsoid in shape and pale in colour as in type (A) spermatogonium, rounded or oval and dark in colour as in type (B) spermatogonium (fig. 15). The primary spermatocytes occupy the middle zone of the germinal epithelium and are recognized by their large vesicular nuclei containing course clumps or thin threads of heterochromatin (fig.12,13). The Sertoli cells rest on the basement membrane of the seminiferous tubules and its cytoplasm extend to the lumen of the tubules, filling the narrow space between the germinal cells(fig. 11,12). The interstitial tissue between the seminiferous tubules containing the Leydig cells in the form of clusters of polygonal cells throughout the interstitium (Fig. 11).

b-Electron microscopic structure of the adult control rate testis shows that, pale type A spermatogonium which is ovoid in shape resting on the basal lamina with large ovoid nucleus having finely granular chromatin, one or two nucleoli attached to the nuclear membrane and some mitochondria in its cytoplasm (fig.14).Dark

type A spermatogonium is similar to the pale type but its nucleus contains patches of heterochromatin along the inner surface of the nuclear membrane (fig. 15). The primary spermatocytes are the largest germ cells, contains central spherical nuclei containing coarse clumps of heterochromatin (Fig. 14) or fine threads of chromatin and its cytoplasm contains peripheral mitochondria arranged near the cell membrane in a signet ring appearance (Fig.16, 17 & 18). The secondary spermatocytes are short lived and therefore rarely seen, it is small in size and their chromatin is less dense. The early spermatids are round cells with spherical nuclei containing finely granular pale stained chromatin and the cytoplasmic organelles are prominent especially Golgi complexes, numerous mitochondria and pair of centrioles (fig.17, 18 & 21). The late spermatids are elongated, found in groups near the lumen of the tubules and formed of head and tail (Fig. 18). The head is directed towards the basal lamina in the cytoplasm of the Sertoli cell and the tail to the luminal direction of the seminiferous tubule. The ultrastructure of the spermatids shows evidences of the spermiogensis which are indicated by appearance of large granule within a sizable acrosomal vesicle in the cytoplasm of the cell near the nucleus which then adheres to the nuclear membrane. The Golgi complex is closely applied to the outer aspect of the vesicle. The granule enlarges and remains at one pole of the nucleus (Fig. 18 & 19). While the surrounding vesicle continues to expands forming a thin fold that extends laterally and posteriorly until it forms a cap over the anterior half of the nucleus (Fig. 20). The nucleus with its cap migrates to one pale of the cell and begin to elongate. The spermatid starts also

to elongate and their centrioles move to the posterior pole of the nucleus, against the nuclear cap, to forms the sperm flagulum. The mitochondria become arranged to form the mitochondrial sheath. The micratubules become arranged to form the nine doublets of the sperms tail (Fig. 21, 22 & 23). The Sertoli cells are recognized by their nuclei which appear triangular or ovoid in shape with homogenous nucleoplasm and rounded nucleolus. Their cytoplasm extends throughout the wall of the tubules, with poorly defined outlines because of the numerous lateral processes that surround the spermatogenic cells and contains numerous mitochondria which arranged parallel to the long axis of the cell and small vacuoles which contain fat dissolve during preparation of the tissue for staining (Fig. 16).

The basal layer of the germinal epithelium and the Sertoli cells supported by a basal lamina which is surrounded by a lamina propria, outside, consisting of the myoid cells which possess a spindle shaped nucleus (Fig. 12 & 24).

The lamina propria in the control rat seminiferous tubule appears thin and regular with deposition of few collagen fibres (Fig.24). The Leydig cells have an oval nucleus with patchs of heterochromatin near the nuclear membrane and their cytoplasm contains numerous mitochondria (Fig. 25), lysosomes, smooth endoplasmic reticulum and many vacuoles of small size where their lipid contents had been extracted during preparation.

Treated group I:

Morphologically: The gross appearance of the rats of this group shows a feminized external genitalia look like an adult female including: Retention of the nipple and areola on the skin of the anterior thoraco abdominal wall after removal or shaving of their hair. A blined vaginal pouch behind the penis (fig.26), which appears abnormal with a cleft prepuce and irregular, lobulated glans penis (fig.27). The length of the ano-genital distance becomes relatively short as compared to the control group with an average length of about 2.3 ± 0.2cm (table 3). The external urethral orifice observed on the under surface of the penis at the junction between the glans and the body of the penis "hypospadius" (Fig.26 & 27).

The testes of twenty two rats of both sides or one side, right or left are not present inside the scrotum but found at the suprainguinal region in twenty rats and found intra-abdominally at the lower pole of the kidney in two rats only (Fig. 28,29,30,31 & 32).

The testes of these groups are smaller than those of the control one, particularly which remained intraabdominal. The average weight of the suprainguinal testes is about 2.97 ± 0.08 gm, and the intraabdominal testes is about 1.32 ± 0.14 gm (table 1 and Fig. 10).

Microscopically:

a-Light microscopic structure of the transverse sections of the seminiferous tubules show varying degrees of degenerative changes ranging from moderate tubular damage in the tubules from the supra-

inguinal testes to sever damage in the tubules of the testes from the intraabdominal one. The seminiferous tubule of the suprainguinal testes shows few number of germ cells with wide intercellular spaces filled with edematous fluids (fig.35). Some of the tubules exhibit sloughing of some germ cells into the lumen of the tubules and rounded bodies can be observed which may represent their debris. The lumen contains degenerating material with no spermatozoa(fig.33 & 34). The basement membrane of the tubules and the interstitial tissue become thick and irregular with high connective tissue (Fig.33). The tubules of the intraabdominal testes shows marked decrease in the number of the germ cells which become nearly single layer, formed of Sertoli cells and spermatogonia only. The lumen contains no sperms but the phagocytic cells appear inside it (Fig. 36).

b-Electron microscopic structure of the seminiferous tubules shows signs of degeneration in some spermatogonia as seen in their nuclear membrane and the cytoplasm contains multiple vacuoles and low number of mitochondria with loss of its cristae (fig.41,43 & 45). The degenerated spermatocytes appear in some fields with shrunken nucleus (Fig.40,37 & 42). The Sertoli cells have minimal number of mitochondria with loss of its cristare and large vacuoles , their nuclei are observed highly lobulated with many deep infolding of its nuclear membrane (Fig.37,38,39, 40 & 44). The basal lamina becomes very thick, irregular and formed of more than two layers with high collagen fibers deposition (Fig.43,48 & 49). The degenerated spermatid is observed in some tubule of moderate

damage(fig.47). The interstitial Leydig cell has pyramidal nucleus, with decreased mitochonderia and increase of its vacuoles (Fig .46).

In the treated group II, there is no marked changes in the testis structures, relative to the control (fig.50,51,52 & 53).

The microscopic structure of the adult control rat epididymis.

The cross section of the adult control rat epididymis shows that, the epithelial lining of the duct of the head of the epididymis is formed of a pseudostratified columnar epithelium, composed mainly of three types of cells, the principal cells, some basal and apical cells (Fig. 55).

The principal cells are characterized by their basal and apical stereocilia that projected into the lumen of the duct. The basal cells are lodged between the bases of the principal cells and not reach the lumen of the duct, but rested on the basement membrane of the duct and possess a triangular shaped nuclei. The apical cells are present between the principal cells but their nuclei lie near the lumen of the duct.

The lumen of the duct is filled by a large number of sperms. The interstitial tissues between the ducts contain smooth muscle fibers and connective tissue cells (Fig. 55).

The epithelial lining of the tail of the epididymis is formed of low columnar to cuboidal principal cells which still have a stereocilia at their apical zone (Fig. 56).

The gross appearance of the epididymis of adult treated male rate group (I) is observed smaller its average weight is about 0.35 ± 0.05 gm and is formed of head, body and tail except that of the intraabdominally located testes which appears as a duct like structure and its average weight is about 0.18 ± 0.03 gm (table 2 and Fig.54).

In the treated rats group I; the epididymis shows degeneration of its epithelial lining with loss of great part of their stereocilia. The smooth muscle fibers and the connective tissue cells in between the ducts becomes degenerated. The lumen of the epididymal ducts contains large number of inflammatory cells in some ducts and degenerated sperms in other ducts (Fig. 57,58 & 59).

In the treated group II, there is no marked changes in the epididymal structures, relative to control.(fig.60).

In the microscopic structure of the adult control rat vas deferens, the duct wall consists of thick muscular tissue arranged in three layers: inner, outer circular and middle longitudinal smooth muscle fibers. The epithelial lining is formed of a pseudostratified columnar epithelium containing centrally located nuclei and apical stereocilia which project into the lumen of the duct. The epithelial lining and their lamina propria are thrown into a longitudinal folds which contain a core of smooth muscle fibers. This produces a small stellat lumen and this permitting expansion of the duct during ejaculation. The sperms are accumulated inside the lumen of the vas deferens (Fig.62).

The gross appearance of the vas deferens of adult male treated rat group (I) shows that the wall of the vas deferens becomes thin in

suprainguinally located testes and very thin in the intraabdominally located testes (Fig.61).

In the treated group I; the epithelial lining of the vas deferens becomes desquamated with loss of great part of its stereocilia which appears free in the lumen of the duct. The lamina propia and the surrounding smooth muscle fibers don't form the longitudinal folds which project inside the lumen of the duct as compared with that of the control group, also there is no sperms in the lumen of this duct (Fig.63). Also shows sever degenerative changes in the vas deferens of the intrabdominal testis as complete loss of its epithelial lining and degeneration of its muscular wall (Fig.64).

The structure of the vas deferens of the treated group II, returns nearly to the control.(fig.65).

The microscopic structure of the adult control rat seminal vesicle, shows a highly coiled and branched acini. The epithelial lining of these acini consists of a pseudostratified columnar epithelium with highly vesicular nuclei and foamy cytoplasm and the cavity of the vesicle contains secretion of these acini. There is also smooth muscular tissue in between the branches of the acini (Fig. 66).

In the treated group I, the seminal vesicle becomes completely degenerated.(fig.29).

The microscopic structure of the adult control rat prostate shows that the prostatic acini are lined by low columnar to cuboidal epithelium and in between the acini a smooth muscle fibers and connective tissue cells. The lumen of some acini contains prostatic

secretion which accumulate to form a prostatic concretions called (corpora amylacea), (Fig.67).

Microscopic appearance of the prostate of adult treated male rate group (I) shows that multiple degeneration of its acini with replacement of the large part of the prostatic acini by a fatty tissues which dissolves during preparation of the specimen. The smooth muscle fibers and the connective tissue in between the acini becomes degenerated (Fig.68).

Effect of the flutamide administration to prost-natal group(II).

The post-natal treatment of rats from birth to post natal day fourteen does not interfere with the development of the external genitalia and all male rats in this group have bilateral descent of the testes into the scrotum.The length of the ano-genital distance is about 3.49 ± 0.3 cm (table 3). The testes are of normal weight and shape with an average weight about 4.05 ± 0.1 gm (table 1). All the internal genital ducts and glands as epididymis (average weight 0.73 ± 0.05 gm table 2), vas deferens, seminal vesicle and the prostate appeared normally as compared to the control group. Histologically the light and the electron microscopic study of the testes shows structures similar to that of the control group. The seminiferous tubules contains all type of germ cells with normal spermatogensis, normal Sertoli cells and the basal lamina appears normal (Fig.50,51,52 &53).

The cross section of the epididymis and the vas deferens have structures similar to that of the control group with no any difference between them. Also the seminal vesicle and the prostatic gland possessed a structures similar to that of the control rats (Fig.60, 65 & 69).

Table (1): Comparison between the weight of the testis of control and treated groups in (gm).

Wt of testis (gm) / Groups	$\bar{x} \pm SD$	t	p
1- Control group (n=20)	4.13 ± 0.09	-	-
2-Scrotal pre-postnatal (n=48)	4.05 ± 0.1	t_1 = 3.23	<0.01
3- Supra inguinal (n=24)	2.97 ± 0.08	t_2 = 44.76	< 0.001
4- Intraabdominal (n=8)	1.32 ± 0.14	t_3 = 52.59	< 0.001

Table (2): Comparison between the weight of the epididymis of control and treated groups in (gm).

Wt of epid.(gm) / Groups	$\bar{x} \pm SD$	t	p
1- Control group (n=20)	0.75 ± 0.08	-	-
2-Scrotal pre-postnatal (n=48)	0.73 ± 0.05	t_1 = 1.04	>0.05
3- Supra inguinal (n=24)	0.35 ± 0.05	t_2 = 19.42	< 0.001
4- Intraabdominal (n=8)	0.18 ± 0.03	t_3 = 27.4	< 0.001

Table (3): Mean and standard deviation of anagenital distance in the studied groups in (cm).

Ano-genital dis.(cm) / Groups	$\bar{x} \pm SD$	t	p
1- Control group (n=10)	3.67 ± 0.19	t_1 = 19.49	< 0.001
2- Treated prenatal (n =30)	2.3 ± 0.2	t_2 = 1.6	> 0.05
3- Treated post (n=10)	3.49 ± 0.3	t_3 = 11.7	< 0.001

t_1 = control us treated pre

t_2= control us treated post

t_3= treated pre us treated post

us = versus

P = 0.05

P > 0.05 : Non significant.

P < 0.05 : Significant.

P < 0.01: Highly significant