The Effects of Progesterone on Mice Offspring Testis Development and Anogenital Distance Postnatally

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Abstract:

Background: Analysis of human reports and comparison with results of experimental animals indicate that the effects of progesterone on human not analogous to experimental animals fetus, many studies showed that exposure to progesterone during developing of genital tract of human fetus was not teratogenic. Other studies which performed on laboratory animals found association between progesterone administration during gestation and genital malformation.

Objectives: to explore the effect of progesterone in 10.2mg/kg intraperitoneal injection in mice on testis development and anogenital distance.

Materials and Methods: ten pregnant mice divided into five mouse control group that injected10.2mg/kg sesame oil and treated group that injected progesterone at dose 10.2mg/kg/day allover gestation to the seventh day postnatally, mice offspring sacrificed, the anogenital distance, weight and diameter of testis were measured ,histological slides were prepared and examined for histopathological change and measurement of seminiferous tubules diameter, germinal epithelium thickness and interstitial space were done .

Results: the current study found anogenital distance decrement and histopathological changes in mice offspring testis as hemorrhage or congestion of blood vessels ,irregular shapes of seminiferous tubules with atrophy, detachment of spermatogenic cells from basement membrane, death of spermatogenic cells and destruction of Sertoli and leydig cells ,it is also showed wider interstitial tissue and thinner germinal epithelium.

Conclusions: The present study indicate study that progesterone injected in mice at dose 10.2 mg/kg /day (the equivalent human dose allover gestation was embryotoxic and teratogenic, it may cause feminization of male offsprings represented by decrement the anogenital distance in mice and hypospadias in human .

Keywords: Progesterone, testis, mice offspring, anogenital distance.

Introduction:

Analysis of human reports and comparison with results of experimental animals indicate that the effects of progesterone on human not analogous to experimental animals fetus, many studies showed that exposure to progesterone during developing of genital tract of human fetus was not teratogenic(1). Other studies which performed on laboratory animals found association between progesterone administration during gestation and genital malformation (2).

The mammals testis is a paired organ that responsible for sperm production sperms by spermatogenesis (3) that is occur in the seminiferous tubules, Sertoli cell helps in spermatogenesis, and activated by Follicle-Stimulating Hormone (FSH) secreted by pituitary gland, interstitial tissue contain Leydig cells that stimulated by the Luteinizing Hormone (LH) and synthesis testosterone hormone, which responsible for secondary sexual characters in the male (4). Progesterone injection lead to decrease conversion of testosterone to the DHT (Dihydrotestosterone), so the testosterone accumulation leading to increase of masculinization of external and internal genitalia in female fetus and decreased in mice male anogenital distance (5) and deviate the sex ratio toward female as well as hypospadias in human male fetus,Dihydrotesterone is known to be the local mediator of penile development (6).

Materials and Methods:

Ten pregnant females mice 8 to 10 weeks age and 25 to 28gm weight, divide equally into control group injected with sesame oil and treated group injected with progesterone 10.2mg/kg/day intraperitoneally from first day of gestation to seventh day postnatally , mothers sacrificed, their male offspring were harvested. Histological slides were prepared, examined for abnormal appearance, measurement of the offspring weight; their testis weight and diameter with anogenital distance were done. Diameters of seminiferous tubules, interstitial space and germinal epithelium thickness were measured.

Results: The mice testis at day seven postnatal of control group showed the tunica albuginea (thick fibrous capsule) on ovoid seminiferous tubules that contain spermatogenic cells arrange on the basement membrane, Sertoli cells, and giant cells and interstitial tissue with Leydig cells (Figure 1, 2).

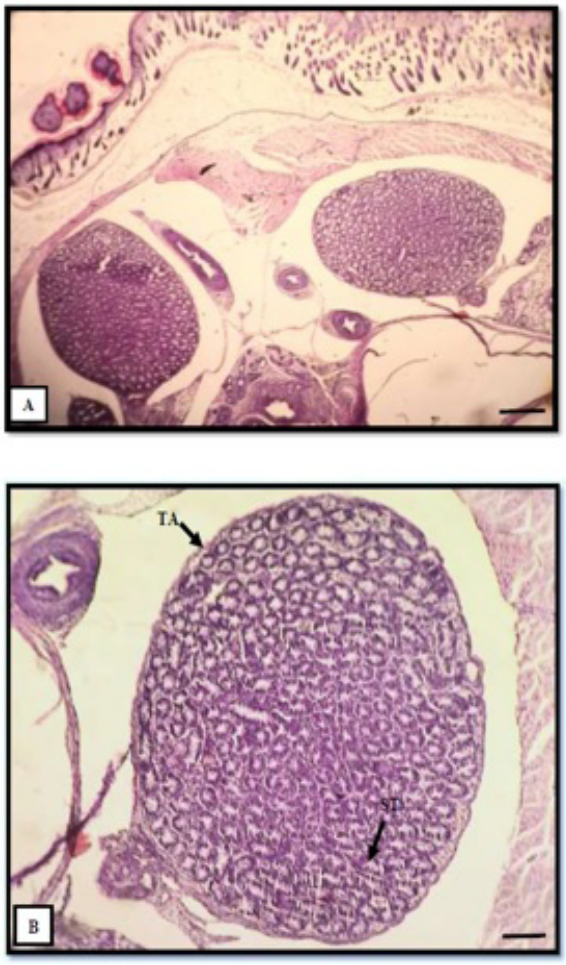


Figure (1): Cross sections through mice testis at day seven postnatal of control group showing tunica albuginea, (TA), seminiferous tubules (ST), (H&E). A: 10X, B: 100X, Scale 100 µm.

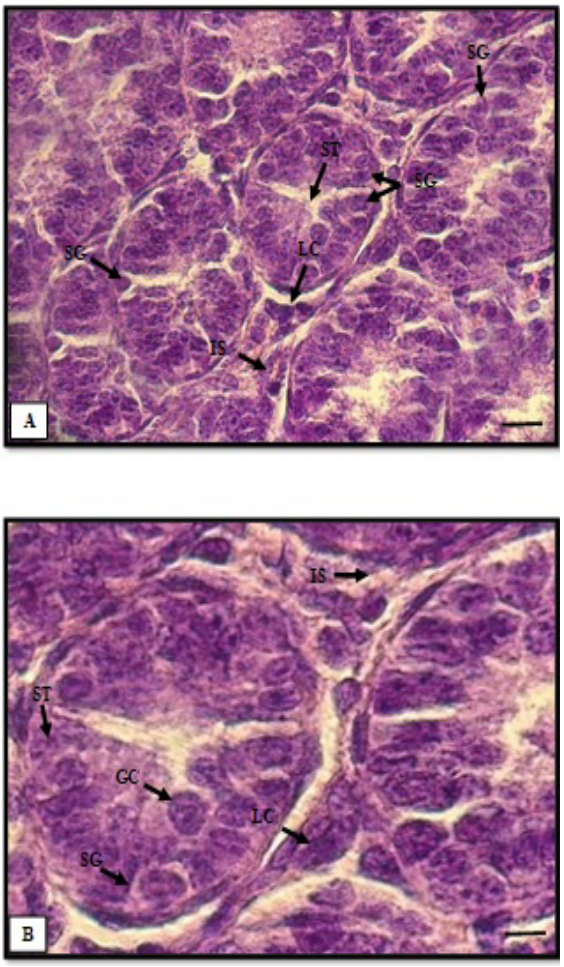


Figure (2): Cross sections through mice testis at day seven postnatal of control group showing seminiferous tubules (ST), Sertoli cells (SC), Giant cell (GC), Leydig cells (LC), interstitial tissue (IS), (H&E). A: 40X, Scale bar: 40 µm; B: 100X, Scale bar: 10 µm.

While treated group showed atrophy and irregular shapes of seminiferous tubules, detachment of spermatogenic cells, congested blood vessels, Hemorrhage, wider interstitial tissue and thinner germinal epithelium (Figure 3,4,5,6).

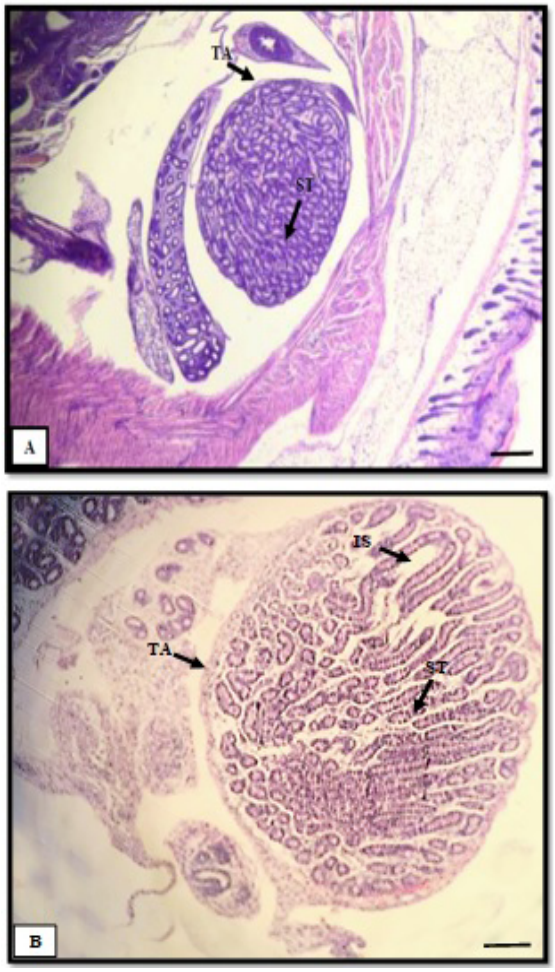


Figure (3): Cross sections through mice testis at day seven postnatal of treated group showing tunica albuginea, (TA), seminiferous tubules (ST), interstitial tissue (IS) (H&E). A: 10X, Scale bar: 100 µm; B: 20X, Scale bar: 80 µm.

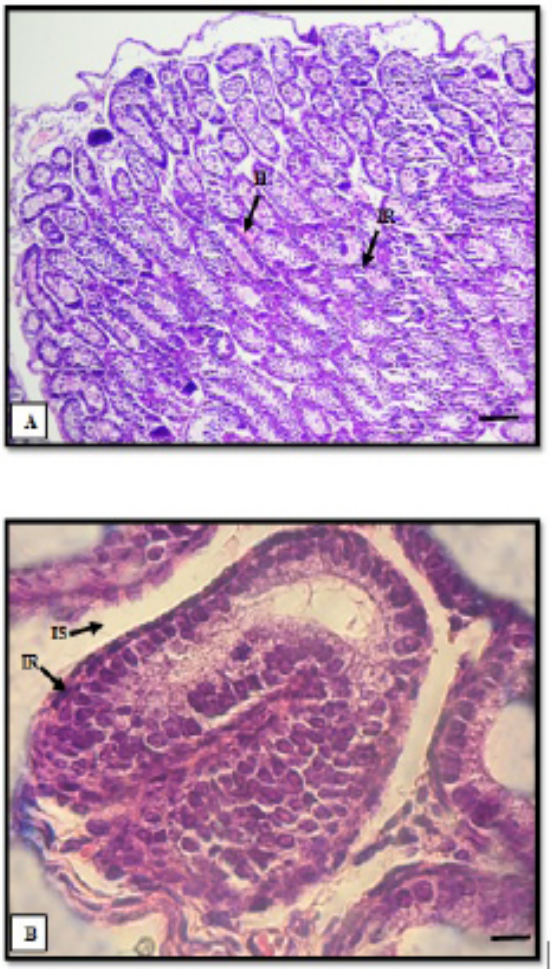


Figure (4): Cross sections through mice testis at day seven postnatal of treated group showing hemorrhage (H), irregular seminiferous tubules (IR) and wider interstitial connective tissue (IS), (H&E). A, B: 40X, Scale bar: 40 µm.

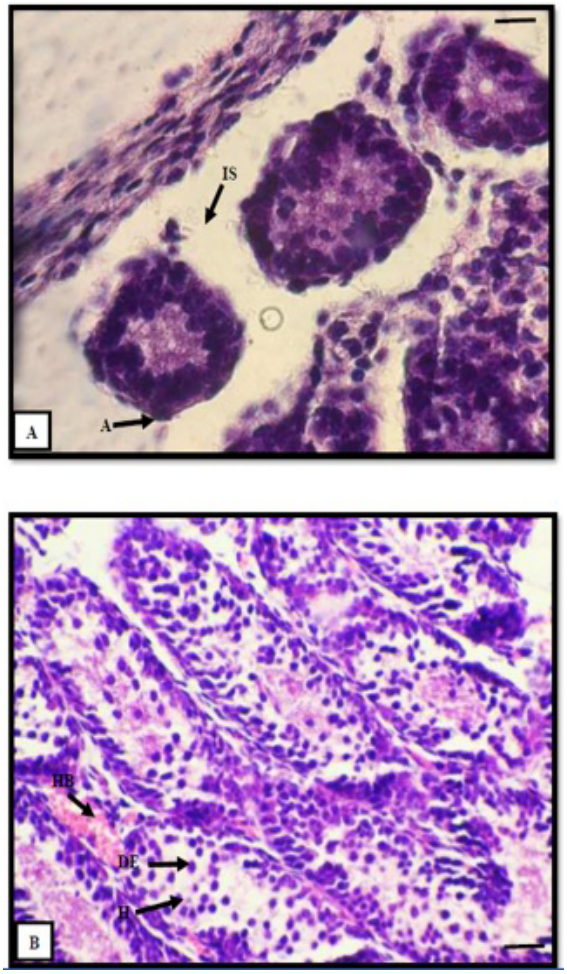


Figure (5): Cross sections through mice testis at day seven postnatal of treated group showing atrophy of seminiferous tubules (A), increased interstitial tissue with reduce connective tissue (IS), congestion of blood vessel (HB), hemorrhage within seminiferous tubules (H), detachment of spermatogenic cells from basement membrane (DE), (H&E). A, B: 40X, Scale bar: 40 µm.

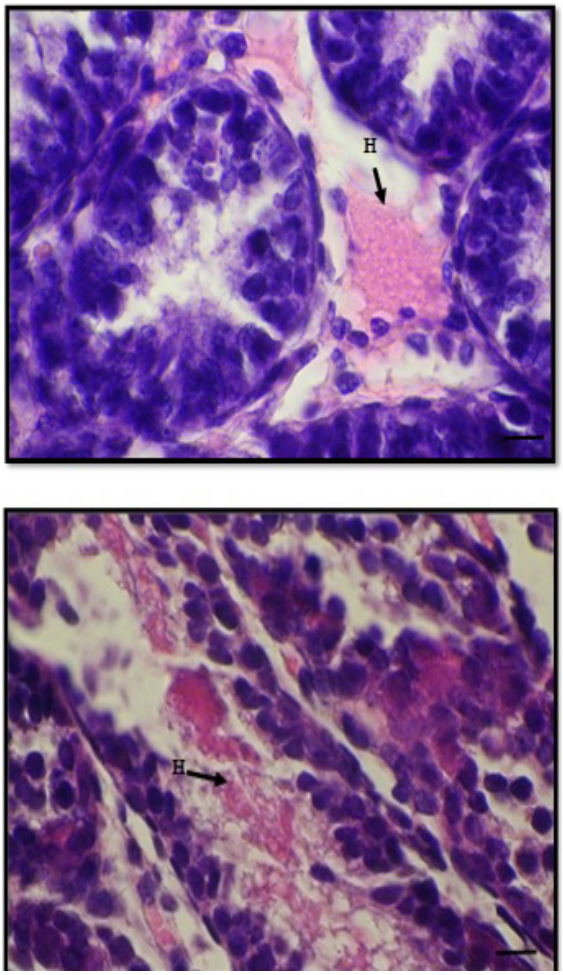


Figure (6): Cross sections through mice testis at day seven postnatal of treated group showing hemorrhage (H), (H&E). 100X, Scale bar: 10 µm.

All the studied parameters except(weight of testis,interstitial space) were decreased in the treated group in comparison to the control one.The difference were decreased significantly in diameter of testis,germinal epithelial height and anogenital distance. While the significant increase was only in the interstitial space, all of these could be ascribed to the main effect of progesterone 10.2mg/kg on the male parameters in mice embryos at 7 day postnatal as summarized in(Table1,Figure 7,8,9,10,11,12,13) as illustrated below.

Table (1) the statistical analysis of mean of the mice male embryo parameter and stander error of control and treated group at 7 day postnatally:

Parameters	Mean±S.E	
	Control group	Treated group
Weight of embryo (gm)	2.522±0.059	2.506±0.08
Weight of testis (gm)	0.116±0.008	0.35±0.243
Diameter of testis (µm)	110.665±0.361	103.07±1.534*
Diameter of seminiferous tubules (µm)	11.481±0.794	9.55±0.536
Germinal epithelial high (µm)	4.676±0.351	3.536±0.234*
Interstitial space (µm)	4.084±0.352	17.373±0.583*
Anogenital distance (mm)	1.849±0.046	1.373±0.153*

* significant differences between control and treated groups

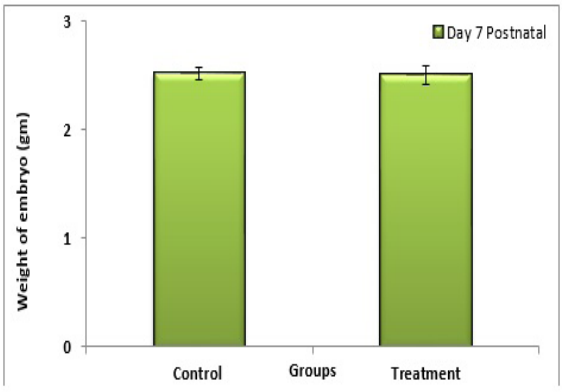
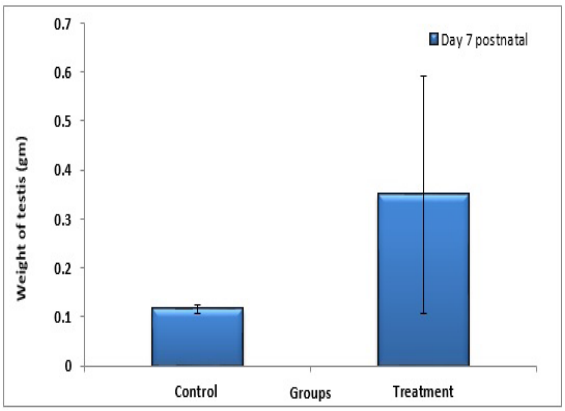
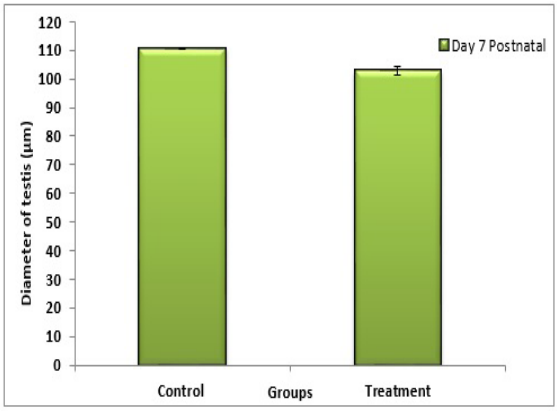


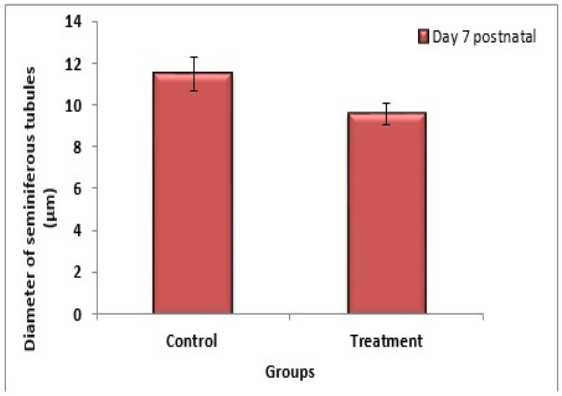
Figure (7): The effect of progesterone (10.2 mg\kg) on mean of weight of mice embryo at 7 day postnatal .



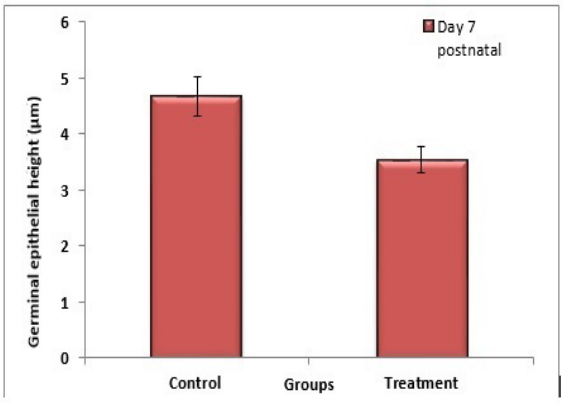
Figure(8): The effect of progesterone (10.2 mg\kg) on mean of weight of testis in mice embryo at 7 day postnatal



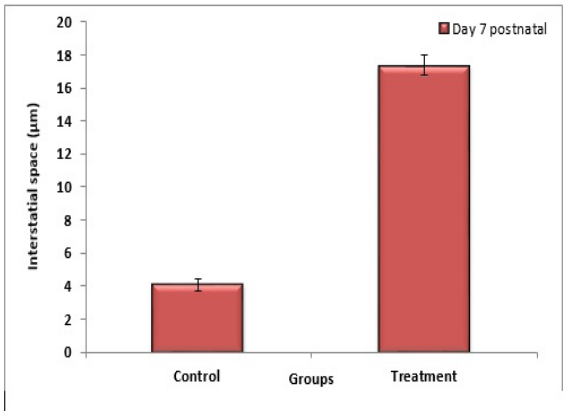
Figure(9): The effect of progesterone (10.2 mg\kg) on mean of diameter of testis in mice embryo at 7 day postnatal . Figure(9):The effect of progesterone (10.2 mg\kg) on mean of diameter of testis in mice embryo at 7 day postnatal .



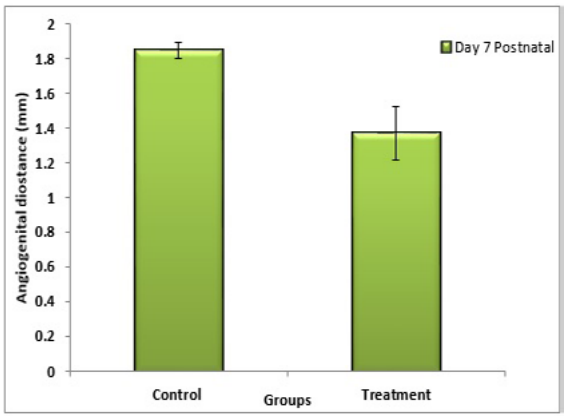
Figure(10): The effect of progesterone (10.2 mg\kg) on mean of diameter of seminiferous tubules in testis of mice embryo at 7 day postnatal .



Figure(11): The effect of progesterone (10.2 mg\kg) on mean of germinal epithelial height in testis of mice embryo at 7 day postnatal .



Figure(12): The effect of progesterone (10.2 mg\kg) on mean of interstitial space in testis of mice embryo at 7 day postnatal .



Figure(13): The effect of progesterone (10.2 mg\kg) on mean of anogenital distance in mice embryo at 7 day postnatal .

Discussion:

At birth the sex determined by anogenital distance (the distance between the anus and urethral papilla) that is greater in male than female (7).

The current study result showed significant decrease in anogenital distance postnatally this result supported other study finding (Silver *et al.*, 1999) (8) which indicate that progesterone was embryo-toxic. measurement of anogenital distance due to effect of progesterone injection during pregnancy feminization of mice males hypospadias in human male and virilization of females (9) due to defect in the activity of the 5a-reductase enzyme or 3 β hydroxysteroid hydrogenase which leads to accumulation of DHEA which require for Testosterone action on the developing male urogenital system (6). The results indicateno significant difference seen neither in weight of mice offspring nor the weight and diameter of their testis these finding agree with previous study accomplished by (Raunig ,JM. 2011) who reviled that the mouse fetus depend on the of maternal cholesterol transfer during pregnancy for normal development(10).

In the present work, the histological sections of the mice offspring testis in treated group showed congestion of blood vessels due to the relaxation and dilatation effect of progesterone injection which lead to inhibition of prostaglandins synthesis that involve in blood flow regulation of testis (11).

The current study revealed destruction of the basement membrane of the seminiferous tubule this corresponds to the finding of Wahbah NS *et al* (12) who indicate that seminiferous tubules basement membrane was disrupted and irregular. The spermatogenic cells detachment from basement membrane of the seminiferous tubules that agree with results of previous study by Yang *et al*. who improve that the detachment of spermatocyte associated with the impairment of meiosis and spermatogenesis (13).

The present study results showed significant decrease in seminiferous tubules diameter(atrophy) in treated groups when compared with control group may be due to destruction of microtubules of seminiferous tubules cells cytoskeleton (14) , another study finding by Adaramoye O. *et al,2013* revealed similar results with decrease in number of tubules(15). A significant increase in interstitial with reduced connective tissue in mice offsprings testis of treated groups this result agree with finding of previous study by Dare BJ. *et al,2012* (16).

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