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EFFECTS OF INSECTICIDE FENITROTHION 40% ON RATS TESTES

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ABSTRACT

Organophosphate (OP) compounds as anticholinesterase agents may secondarily act on diverse serine hydrolase targets, revealing unfavorable physiological effects including male reproductive toxicity. Exposure to Fenitrothion 40% (FNT) insecticide in agriculture and public health has been reported to affect male reproductive organs, thereby leading to spermatotoxicity in rats. To investigate the acute oral toxicity dose of FNT, adult and young rats were subdivided into 11 groups with ten animals each. Ten graduated doses were given orally to 10 groups of rats for the determination of LD_{50}. The effects of Fenitrothion on the body and testes weight, biochemical and oxidative stress, sperm characteristics and histological changes in the testes were evaluated for different periods 14, 28, 42 days at doses 1/20 and 1/60 of FNT. Our results showed that FNT significantly decreased the body weight gain and weight of testes compared with the control group. Fenitrothion altered the sperm characteristics, such as sperm concentration, sperm viability and normal sperm morphology as well as degeneration of germ cells, expansion of interstitial space, disarrangement of spermatogonia in seminiferous tubule and degeneration of Leydig cells compared with the control group. These results suggested that Fenitrothion induces impairments of the seminiferous tubules structure and spermatogenesis in the rats and therefore damages of the male reproductive system.

Keywords

Fenitrothion, young rats, adult rats, histopathology, testis, Spermatogenesis.

Introduction

Organophosphate insecticides represent one of the most widely used classes of pesticides with high potential for human exposure in both rural and residential environments (**korany and Ezzat,2011).**). Fenitrothion [O,O-dimethyl-O-(3-methyl-4-nitrophenyl) phosphorothioate] (FNT) is a broad-spectrum organophosphate insecticide that distresses the nervous system by inhibiting acetyl cholinesterase activity (**Sarikaya *et al.*, 2004**). It is employed in agriculture to control insects and mites that affect cereals, rice, fruits, vegetables, stored grains and cotton (**Uygun *et al.*, 2005**). FNT also used as a fly, mosquito and cockroach residual contact spray for farms and public health programs (**Sarikaya *et al.*, 2004**). Humans are potentially exposed to FNT either directly through occupational exposure or indirectly via food consumption. Unfortunately, like many pesticides, FNT has been classified on the ‘‘red list’’ by the United Kingdom Environment Agency as one of the most dangerous substances to the aquatic environment (**Crathrone and Dobbs, 2001**). The FNT can produce some toxic and adverse effects on liver, kidney, thyroid gland and other biological systems when tested on various types of experimental animals (**Poovala *et al.*, 1999; Kovacic *et al.*, 2003**). FNT acts also on the endocannabinoid signaling system in male reproductive organs, causing spermatotoxicity and testicular damage in experimental animals **(Ito *el al.*, 2014; Taib *et al.*, 2013**). Previous research has found that various concentrations of FNT caused histopathological effects on the liver and kidneys of rats, cytotoxic effects on the lungs of rats and immunosuppressive effects in rats (**Elhalwagy *et al.*, 2008**). In light of this background, the recent study was conducted to investigate the effect of Fenitrothion 40% that has been used in the Kingdom of Saudi Arabia on rats' testes tissues and identify the oral half-lethal dose 50 (LD50) and an acute, and sub-acute doses for young and adult rats.

Methods and materials

Chemical

Fenitrothion insecticide is used in the formulated form, which contains Fenitrothion 40% in addition to 60% oil soap solute. FNT was purchased from the Arabian farms in Jeddah (Saudi Arabia), CAS no (118-141-2324).

Acute oral toxicity

Healthy 110 adult Wistar rats were divided into eleven equal groups each containing ten animals and One hundred and ten (110) young Wistar rats were also divided into eleven equal groups each containing ten animals. 24h after acclimatization, ten graduated doses of Fentroth EC (Fenitrothion 40%) (300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200 mg / kg of body weight /mg/kg/bw) were given orally to 10 groups of rats for the determination of LD_{50} of the combination starting from 0% mortality to 100% mortality (table 1) (**Randhawa.,2009**). 24h after administration of pesticide, animals were observed for respiratory and CNS symptoms, behavioral changes and death. LD50 was determined as per the method of **Miller and Tainter (1994).**

Animal treatment schedule

A total of 90 healthy adult and young male Wistar rats (*Rattus norvegicus*) obtained from King Fahd center for Scientific Research, King Abdel-Aziz University, Jeddah, Saudi Arabia. The rats were maintained on standard laboratory rodent diet pellets and were housed in humidity and temperature under standard environmental conditions on a 12 h day/ night cycle throughout the study period. After determination of LD50 of Fenitrothion, two sub-acute doses as follows: a low dose equal to 1/60 of LD50 and a high dose equal to 1 / 20 of LD50,

according to LD50 values for the pesticide among young and adult rats. 45 young rats and 45 adult rats were divided into 6 groups. Adult rats were divided into 3 groups .Group (G_1), control-group, included 15 rats divided into 3 equal subgroup, five rats each, received distilled water. Group (G_2), divided into 3 subgroup of five rats each, received Fenitrothion 40% (1/60 of LD50). Subgroups G_{2a}, G_{2b}, G_{2c} received the same dose of FNT for 14, 28 and 42 days, respectively. Group (G_3), divided into 3 subgroup of five rats each, received Fenitrothion 40% (1/20 of LD50). Subgroups G_{3a}, G_{3b}, G_{3c} received the same dose of FNT for 14, 28 and 42 days, respectively. This process was repeated for young rats.

Histological study

The rats were sacrificed by cervical decapitation at intervals of 14, 28 and 42 days, and the testes samples were extracted and fixed in neutral buffered formalin (10%), dehydrated and dehydration was then followed by clearing the samples in xylene ,then impregnated with paraffin wax, then embedded and blocked out. Paraffin sections (μ um) were stained with hematoxylin and eosin according to **Bancroft and Gamble (2008).**

Morphological and histological measures

Optical microscope sections of tissue stained by hematoxylin and eosin were examined using eye piece micrometre and oil emersion lens (100x). Different diameters of seminiferous tubules and heights of germ cells layer, the diameters of the Leydig's cells were measured, in the testes among treated rats and control groups by measuring ten microscopic fields randomly. The median and standard deviation of measurements were calculated according to **Hummdi ,(2012)** method.

Statistical analysis

The differences between the treated and the control groups were statistically evaluated using student t-test and Chi-square test. All data are expressed as the mean values ±SD, with significant values at $p<0.05$ and $p<0.001$.

Results

Bioassay of Fenitrothion 40%

Acute toxicity

For adult rats, mortality is observed when the dose of Fenitrothion is 500 mg/kg. Fenitrothion did not induce any effect at 300mg/kg for young rats but at 1000 mg/kg all rats died. The acute oral LD50 value was calculated as 619.02 mg/kg BW for young rats and 790.27 mg/kg BW for adult rats (table 1, table 2).

Sub-acute toxicity

Selected sub-acute low dose equal to 1/60 of median lethal oral dose (LD50) of Fenitrothion for young male and adult rats, and another sub-acute high equal to 1/20 of median lethal oral dose to administrate to young and adult rats, (Tables 3) illustrate the values of the low and high dose of Fenitrothion given daily for young, adult rats and the values of cumulative doses at the end of each experimental period (14,28,42 days).

Signs of toxicity

Administration of high dose of FNT to adult and young rats caused cholinergic signs, such as hypo-activity, lacrimation, piloerection and tremor. By extending the treatment period, some rats death mostly in group C. Results in table 3 revealed that young rats were more sensible for Fenitrothion compared to adult rats.

Body and testes weights

The body and testes weight of the FNT and control groups are provided in Table 4 and 5. The young rats treated with FNT (G_3, G_2) showed weight-gain decrease compared with the control group (G_1) and this decrease was high significantly in the group (G_3). The relative weight of the young rats testes was significantly higher in the control group than FNT group. The most significant decrease in tests weight of young rats testes of groups (G_2b,c, G_3b,c) was observed after 28 and 42 days of treatment compared with other groups. The table (5) shows a significant decrease in average of body weight among adult rats treated with low and high doses (G_3, G_2) after 42, 28, 14 days, respectively, compared to the control group (G_1). However, the table shows a significant increase in mean relative weight of testes among adult rats treated (G_3, G_2). This is followed by a significant decrease in weight of testes with extension of treatment duration compared to control group;

Histological examination:

The normal histological structure of rat testes is illustrated in (Plate 1a,d). Cross-section among control rats testes age of 36 days (G_{1}**a**) shows an increase in average diameters of seminiferous tubules (177.3 ± 15.18 μm) and the emergence of inner lumen inside of seminiferous tubules. Germinal epithelium is composed by spermatogonia and round spermatids in distinct cellular associations; thickness of germinal epithelium is 65 ± 5.47 μm. Primary spermatocytes are characterized by a big size and contain large nucleus with average diameter of 6.01 ± 0.4 μm it is characterized by leptotene or zygotene or pachytene in meiosis prophase I. Secondary spermatocytes are characterized by circular nucleus containing homogeneous chromatin with average diameter of 4.9 ± 0.89 μm sometimes we note a centralization of chromatin. Early spermatids are characterized by abundant granular cytoplasm and central nuclei containing on chromatin bare and with average diameter of 5.88 ± 1.04 μm (Plate 1a). Late spermatids appears around the internal cavity of seminiferous tubules among rats aged 50 days (**G1b**); (Plate 1b,c) illustrates mature spermatids direction in germinal epithelium toward Sertoli cells and some of seminiferous tubules contains spermatozoa. Examination of young rats control group testes at age of 64 days (G_{1}**c**) made clear sexual maturity where seminiferous tubules get longer and increase in size in order to reach the average μm 592 ± 63.79 in length and μm 290 ± 10.0 in width also observation of tubules expansion and become filled with mature sperm. Administration of FNT at a low dose for young rats (group G_{2}**a**) showed a similar histology to control group. Many healthy spermatocytes which are characterized by natural nuclei and gets rushed to the tubules cavity, we notice also necrosis of some spermatocytes, which was characterized by pyknosis with separation from germinal epithelium and gets rushed to the tubules lumen in addition to decomposition of fibrous connective tissue surrounding the tubules and congestion of interstitial tissue blood vessels (Plate 2a). In spite of, with increasing duration of treated category (G_{2}**b**) maturation of spermatocytes and spermatids in seminiferous tubules as found in normal testes in a young group were observed, the separation and sloughing of spermatocytes continued in addition some spermatids gets rushed to the tubules lumen(Plate 2b). With increasing duration of treated in category (c) we noticed decreasing in germinal epithelium in many tubules and vacuolar degeneration of spermatogonia while Sertoli cells remain clear (Plate 2c,d). (Plate 2d) illustrates a severe disabilities in the process of spermatogenesis in varying degrees " hypospermatogenesis" and lack of sperm in most of

seminiferous tubules and emergence of multinucleated giant cells. in addition to an increase in cellular content and fibrous tissue with proliferation of blood vessels "angiogensin", oedema and inflammatory cellular infiltration in the interstitial tissue. Examination of testes sections of the group (G3) treated with high dose category (a) made clear a decomposition of fibrous connective tissue surrounding seminiferous tubules and congestion of interstitial tissue blood vessels and disturbance in germinal epithelium lining seminiferous tubules (Plate 3a) illustrates some tubules containing normal germinal epithelium with lack of spermatocytes "hypospermatogenesis" and infiltration of blood fluids into others and decomposition of spermatids. Increasing the duration of treatment in category (b) made clear in some sectors severe deficiency of cells in result to cellular necrosis where cells gets detached from connections and nuclei degenerate leading to shortages and trouble in germinal epithelium tubules addition to non-appearance of mature spermatids(Plate 3b). However we observed normal growth and maturation in some other seminiferous tubules from other sections. The examination of sections (Plate 3c) from testes treated in category (c) indicates heterogeneity in seminiferous tubules histological damages showing a general reduction of seminiferous tubules size (211.5 ± 14.129 μm in length 140 ± 12.116 μm in width) and absence of mature spermatozoa compared to control group. as we observed deformation and atrophy of some tubules characterized by stop of spermatogenesis process "aspermatogenesis" as we noted the decomposition of the entire spermatids and some spermatogonia while nuclei of Sertoli cells remain clear and intact in some tubules and decreases in number and getting smaller in other tubules (Plate 3d), this in addition to a connective interstitial tissue increase and cellular infiltration followed by significant aneurism and coagulation (Plate 3c,d). Above mentioned changes was committed to significant decrease in testes weight compared to control group (Table 4).

On the other hand, The examination testes sections among the adult group (G2) treated with low dose category (a) we notice the same results were observed in young rats. While, it was observed in other sections infiltration of blood fluids in interstitial tissue, edematous testes, cellular infiltration and lack of mature spermatozoa, accumulation of residual bodies and formation of multinucleated cells (Plate 4a). With increasing duration of treatment in category (b) we noted the absence of spermatids in cavity of many seminiferous tubules which become distorted and filled with dead spermatozoa, healthy spermatozoa and sloughing mature spermatids in addition to necrosis in many spermatogonia adherent basement membrane however Sertoli cells remained clear and distinct, also notify atrophy of some tubules with appearance of decomposition in Leydig cells consisting on hypertrophy of some nucleus, pyknosis of some other and congestion of blood vessels in interstitial tissue (Plate 4b). Histological damages intensify in testes with extension of treatment duration category (c) where several seminiferous tubules expand and shatter and stops spermatogenesis process, also we notes emergence of large gaps as result of spermatids necrosis and observed disorder in germinal epithelium and accumulation of residual bodies, atrophy of tubules with an increase in basement membrane thickness, RBCs accumulates in blood vessels of interstitial tissue (Plate 4c,d).

The histological examination of the testes Group (G3) treatment dose-high category (a) showed absence of mature spermatozoa in most of tubules and that cavities are filled with spermatids and spermatocytes dead and sloughing, while we observed germinal epithelium retaining somehow its morphological aspect (Plate 5a). Blood vessels congestion and

infiltration of blood fluids in interstitial tissue while Leydig cells remained normal were observed. With extension of treatment duration in category (b), absence of spermatozoa and mature spermatocytes in addition to the critical expansion of some tubules containing atrophied, necrosis and decomposed spermatozoa cells (Plate 5b,c) were observed. Necrosis and decomposition in many spermatogonia but Sertoli cells remained clear (Plate 5c). An increase in basement membrane thickness and volume of interstitial tissue. Histological damages intensify in rats testes with extension of treatment duration in category (c) where the spermatogenesis process stops in tubules and its diameters decreases compared to control group 202.314 ± 20.641 μm ($P < 0.001$) as many tubules deform, atrophy and notes decomposition of the interstitial tissue in order Sertoli cells decrease in number and volume with blood vessels fibrosis of interstitial tissue (Plate 5d). Also observes tubules containing decomposed and dead cells as we noticed decomposition in many spermatogonia and Sertoli cells with tubular lysis (Plate 5d) in addition to testicular atrophy committed to a significant decrease in men weight compared to control group (Table 5).

Discussion

Exposure to organophosphate is a health risk for living organisms, including human. Humans can be exposed to these toxic compounds either due to occupational exposure or accidental consumption of contaminated food (**Uzun ,2009**). The current toxicity scenarios have attracted the attention of researchers studying the toxic effects of organophosphate on the male reproductive system. FNT has been found to cause some reproductive abnormalities with a reduction in the sperm quality (**Kamijima , 2004**). It has become among the etiologies contributing to male infertility (**Ben Abdallah ,2012**). Results of the present study demonstrate that the acute oral LD50 value was calculated as 619.02 mg/kg BW for young rats and 790.27 mg/kg BW for adult rats. This study showed that subchronic FNT administration produced toxicity in adult and young rats as monitored by decrease in body weight gain and increase in testes weights in rats. In addition it was observed that a significant decrease in body weight gain and in testes weight was observed in the group treated with high dose. The reduced weight gained could be attributed to systemic toxicity in male rats (**Mylchreest *et al.*, 2006**). The significant reduction in the weight of the testes in adult and young rats treated with high dose of FNT cause a mild decrease in sperm concentration, motility, and morphology (**Okahashi, 2005**; **Okamura, *et al.*, 2009**). In addition a significant decrease in weight of testes with extension of treatment duration 14, 28, 42 days) compared to control group were observed. Many previous studies has recorded significant decrease in testes weight, seminal vesicle, prostate gland among rats and mice treated with pesticides (**Debnath and Mandal, 2000; Tamura *et al.*, 2001**). Our histological examination showed that the FNT exposed groups illustrated by histopathological changes in sperm and testis compared with control group. In fact, histological structure of rats control testes (G_1) is similar to normal testes of other mammal. However, the current study indicates reproductive toxicity of phosphoric organic pesticide Fenitrothion on young and adult rats treated with non-acute low and high dose of the pesticide during experimental period (6 weeks), where pesticide made changes in spermatogenic cells such as necrosis in spermatogonia, spermatids, spermatocytes and stop of spermatogenesis process. Extension of treatment duration with repeated dose lead to necrosis of spermatogonia and the absence of germinal cells from tubules, which leads to atrophy of seminiferous tubules and increase the interstitial connective tissue, which causes atrophy of testes and testicular dysfunction among adult rats and hampers sexual maturity and puberty among young rats . Our findings are

generally consistent with the studies by **Izatus *et al.*, (2013)** who reported that FNT caused pathological changes in seminiferous tubules following a 28-day exposure**.** Emergence of multinucleated giant cells, which may be due to fusion of spermatocytes and spermatids which shows signs of decomposition and cell death (**Salem *et. al.*, 1996). Piña-Guzmán *et al.*, (2009)** noticed decreasing in germinal epithelium in many tubules and vacuolar degeneration of spermatogonia while Sertoli cells remain unaffected. **Junqueira and Carneiro,** (**2005**) stipulated that Sertoli cells are resistant to harmful factors such as infection, lack of nutrition, X-ray and survives better than other germinal cells similarly **(Yousef, *et. al.*, 1996**) concluded that Sertoli cells remained unaffected in the testes of rats treated with methiotrxete even if germ cells were destroyed. **Moreover,** current study is consistent with previous studies where **Saxena and Mani (1987)** noted a decrease in testes weight and stops formation of mature spermatocytes in fresh water murrel of rats treated with non-acute repeated doses of Fenitrothion. The extension of treatment period leads to necrosis of spermatogonia and primary spermatocyte and the absence germinal cells and formation of fibrous around dead cells. Study by **Tamura *et. al.*, (2001)** has shown interference of Fenitrothion with androgen hormone receptors in testes of rats treated with non-acute dose of pesticide (30.15 g / kg of body weight) leading to a lack of testosterone which responsible for maturation of spermatocytes, causing its sloughing and death. Many studies proved the death of spermatocytes and sloughing of healthy cells, immature spermatids and seprmatozoa in testes of animals treated with non-acute repeated dose of pesticide (**Salem *et. al.*, 1996; Elbetieha *et. al.*, 2001; Okahachi N *et al.*, 2005). Kamini *et al*, (2014)** attributed the sloughing of healthy cells to the lack of follicle stimulating hormone (FSH), while **(Guraya, 2011)** attributed it to inhibition of formation the occluding junctions between the lateral walls of adjacent Sertoli cells, which leads to cellular disturbance and inhibition of secondary spermatocytes mitosis. The adult rats showed the same results with histological damages in seminiferous tubules, Sertoli cells and stops spermatogenesis process (**Okahachi *et al.*, 2005**). Examination of testes sections in present work agree with **Trivedi *et al.*, (2010)** study who revealed that treated high dose causes "hypospermatogenesis" and infiltration of blood fluids into others and decomposition of spermatids for young rats and showed absence of mature spermatozoa in most of tubules for adult rats. In recent work histological damages intensify in rats testes of adult and young rats with extension of treatment duration in category (c) where deformation and atrophy of some tubules and its diameters decreases compared to control group were correspond with **Creasy , (2001)** work. Study performed by **Turner *et al.*, (2002)** supported our findings, who found that FNT inhibited the maturation and differentiation of germ cells. Toxicants can directly disturb the function of Sertoli cells, thereby causing the disorganization of germ cells in the seminiferous tubule of the FNT-treated group**.**

Table (1) – The percentage mortality recorded 24 hr after Fentroth EC (40% Fenitrothion) treatment in young and adult male rats.

Dose mg/kg BW	Youngs			Adults		
	N. of individuals	Mortality		N. of individuals	Mortality	
		N	%		N	%
300	10	0	-	10	0	-
400	10	1	10	10	0	-
500	10	3	30	10	1	10
600	10	5	50	10	2	20
700	10	6	60	10	3	30
800	10	7	70	10	5	50
900	10	9	90	10	6	60
1000	10	10	100	10	8	80
1100	10	10	100	10	9	90
1200	10	10	100	10	10	100
Control	10	0	-	10	0	-

Table (2): Acute toxicity of Fenitrothion to young and adult male rats.

Treated animals	Fenitrothion
	24 hr LD50 (mg/kg BW)
Youngs	619.02 (594.71 – 644.33)*
Adults	790.27 (764.98 – 816.40)*
Sign. test	χ^2 = 5.42589; P=0.24632

* Values in parenthesis are the 95% confidence limits

χ^2 = Chi square test; P>0.05 not significant

Table (3): Times – Dose schedule for Fenitrothion treated animals.

Young groups	No. of animals	Dose (mg/kg/BW per day)	subgroups		Period of exp. (days)	Cumulative dose	Mortality	
			Abbrev.	N			N	%
G1 control	15	-	**G_1a**	5	14	-	-	
			G_1b	5	28	-	-	
			G_1c	5	42	-	-	
G2 Low dose	15	**10.3171 mg/kg B.W./day**	**G_2a**	5	14	**144.4394**	-	
			G_2b	5	28	**288.8788**	1	20
			G_2c	5	42	**433.3182**	2	40
G3 High dose	15	**30.9512 mg/kg B.W./day**	**G_3a**	5	14	**433.3182**	1	20
			G_3b	5	28	**866.6336**	1	20
			G_3c	5	42	**1299.9504**	2	40
Adult	No. of	Dose	subgroups		Period of	Comulative	Mortality	

groups	animals	(mg/kg/BW per day)	Abbrev.	N	exp. (days)	dose	N	%
G1 control	15	-	**G_1a** **G_1b** **G_1c**	5 5 5	14 28 42	- - -	- - -	
G2 Low dose	15	**13.1712 mg/kg B.W./day**	**G_2a** **G_2b** **G_2c**	5 5 5	14 28 42	**184.3968** **368.7936** **553.1904**	- - 1	 20
G3 High dose	15	**39.5136 mg/kg B.W./day**	**G_3a** **G_3b** **G_3c**	5 5 5	14 28 42	**553.1904** **1106.3808** **1659.5712**	1 1 2	20 20 40

Table (4): Mean body Weights and mean relative weights (R) of testes for control and Fenitrothion treated young male rats at the end of each experimental period.

Body and organ weights **Sub-groups and treatment days**		**Body weight (g)**			**Relative testes weight (g)**		
		Control G_1	**treated**		**Control G_1**	**treated**	
			G_2 Low dose	**G_3 High dose**		**G_2 Low dose**	**G_3 High dose**
	Avg.	32.500	34.000	32.000	1.0985		
	± SD	0.408	0.816	0.408	0.012		
0	T		-2.3238	1.2247			
	P		0.0808	0.2879			
	Avg.	119.500	106.33	87.833	1.732	1.717	1.651
(a)	± SD	2.041	5.307	2.656	0.06611	0.12962	0.14511
14	T		2.46641	13.368		0.8951	4.228
	P		0.10295	0.0002**		0.2326	0.0133*
	Avg.	156.500	148.500	128.500	1.789	1.6869	1.479
(b)	± SD	1.225	5.307	5.307	0.11831	0.1482	0.5621
28	T		2.07716	7.2701		3.4136	12.7764
	P		0.10636	0.0019**		0.0465*	0.00301**
	Avg.	206.500	196.82	180.500	1.7192	1.6429	1.2557
(c)	± SD	0.408	5.225	1.225	0.09111	0.00921	0.1241
42	T		1.5938	28.4816		3.1722	9.4608
	P		0.1862	9E.06**		0.04333*	0.0007**

Significant levels: - $p > 0.05$ not significant

- $P < 0.05$ * significant; $p < 0.01$ ** or < 0.001 ** highly significant

T-student t-test; ± SD : standard deviation

Table (5) : Mean body weight and mean relative weights (R) of testes for control and Fenitrothion treated adult male rats at the end of each experimental period.

Body and organ weights Sub-groups and treatment days		Body weight (g)			Relative testes weight (g)		
		Control G_1	treated		Control G_1	treated	
			G_2 Low dose	G_3 High dose		G_2 Low dose	G_3 High dose
	Avg.	194.500	195.667	195.000	1.993		
	± SD	1.225	1.247	1.871	0.042		
0	T		-0.9439	-0.3162			
	P		0.39868	0.76764			
	Avg.	222.333	204.000	183.000	1.732	1.922	2.077
(a)	± SD	6.128	0.816	4.899	0.06611	0.09641	0.07113
14	T		4.19371	7.08993		3.6742	5.23723
	P		0.01377*	0.00209**		0.02131*	0.00635**
	Avg.	238.833	216.500	199.000	1.654	1.601	1.323
(b)	± SD	1.027	0.408	6.532	0.01006	0.1083	0.11088
28	T		28.5689	8.51943		4.0929	9.4608
	P		8.9E.06**	0.00104**		0.0114*	0.008**
	Avg.	253.167	232.000	246.833	1.659	1.499	1.228
(c)	± SD	1.929	3.674	9.784	0.01269	0.05932	0.01142
42	T		7.21312	4.6073		4.899	9.4618
	P		0.00196**	0.0067**		0.0081**	0.0008**

Significant levels : - $p > 0.05$ not significant

- $P < 0.05$ * significant ; $p < 0.01$ ** or < 0.001 ** highly significant

T-student t-test; ± SD : standard deviation

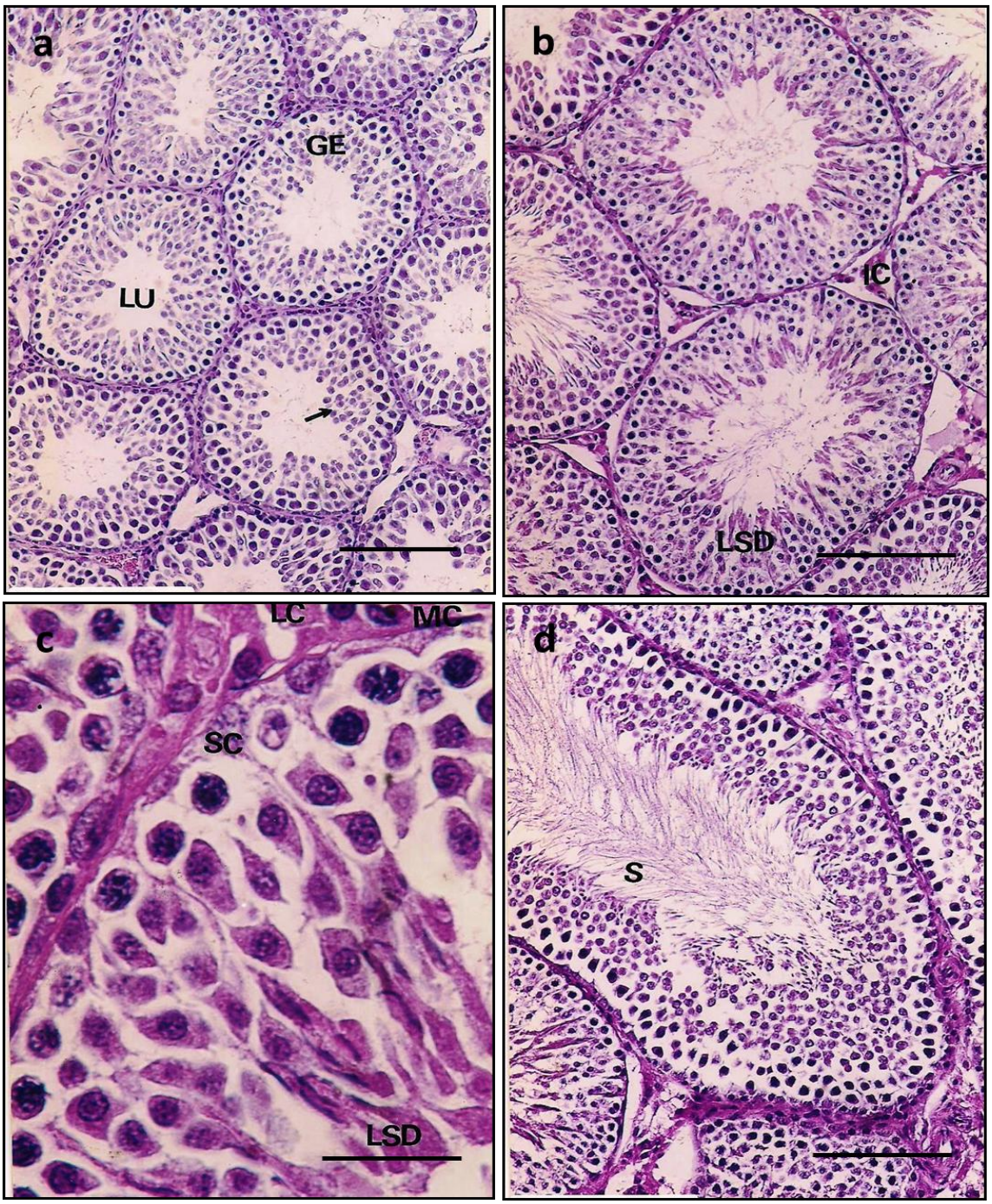
a
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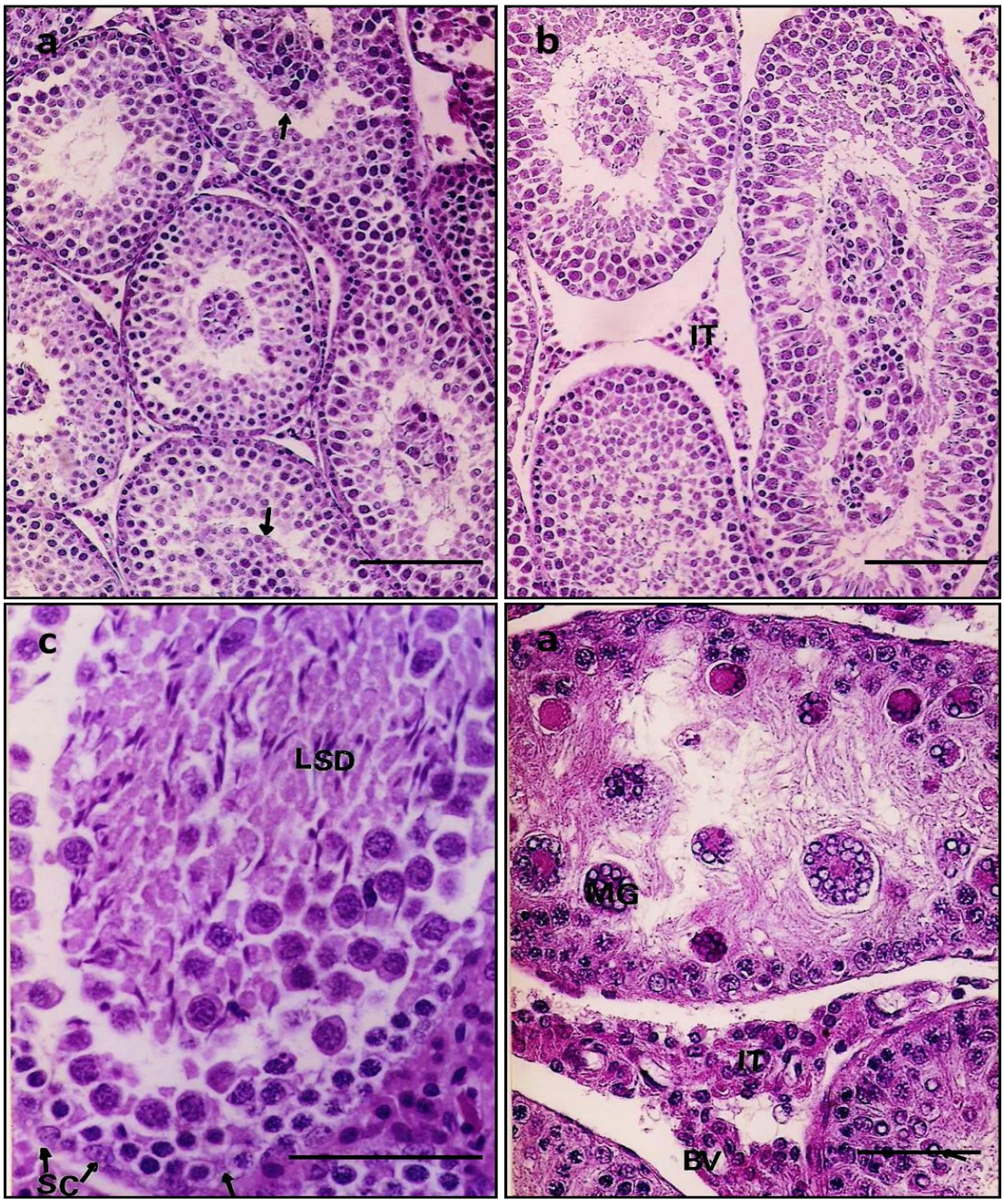
Plate (1a-d) : L.M. testicular cross sections of control young and adult groups of Wister rats (H. & E.). (a) section showing of control young group (G_{1a}) at 36 days age Notice tubular basal lamina (BL) with sertoli cell (SC) & spermatogonia (SG); spermatocytes and early spermatids (ESD). Note interstitial tissue (leydg's) cells (LC); scale bar = 20 μm. (b) Section of control young group (G_{1b}) at 50 days age Notice Enlarged seminiferous tubules populated by spermatocytes and late spermatids (LSD) surrounded the tubular lumen. Note wide intertubular spaces contain interstitial cell (IC); scale bar = 100 μm. (c) High power from previous section (1b) showing late spermatid (LSD) with elongated head directed towards sertoli cells (SC). Note myoid cell (MC) & interstitial leydg's cells (LC); scale bar = 20 μm. (d) Section of control adult group (G_{1c}) at 64 days age showing full maturation of testicular tissue. Note elongated seminiferous tubules with regular arrangement of germ cells and wide lumen full of spermatozoa (S); scale bar = 100 μm.



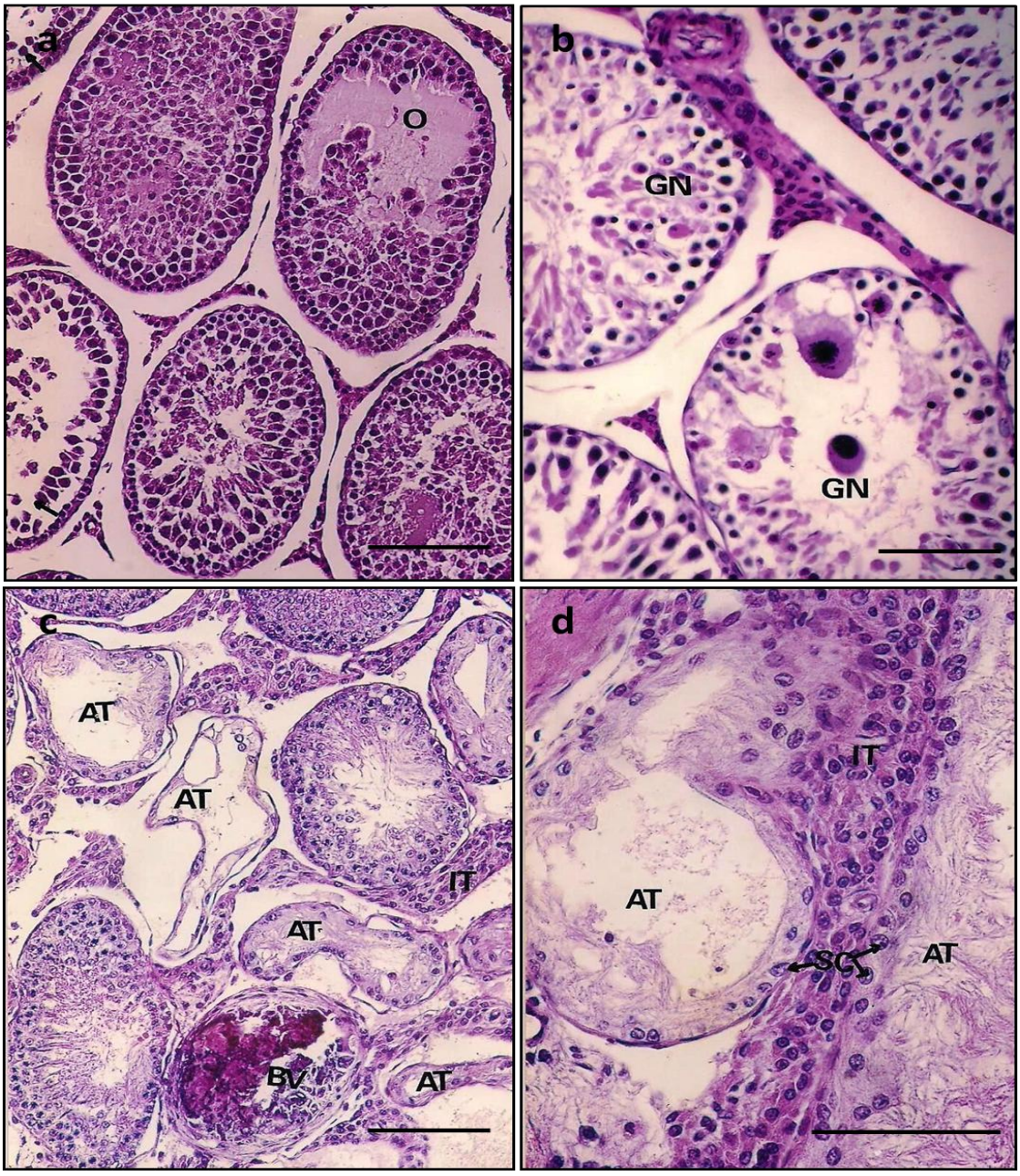
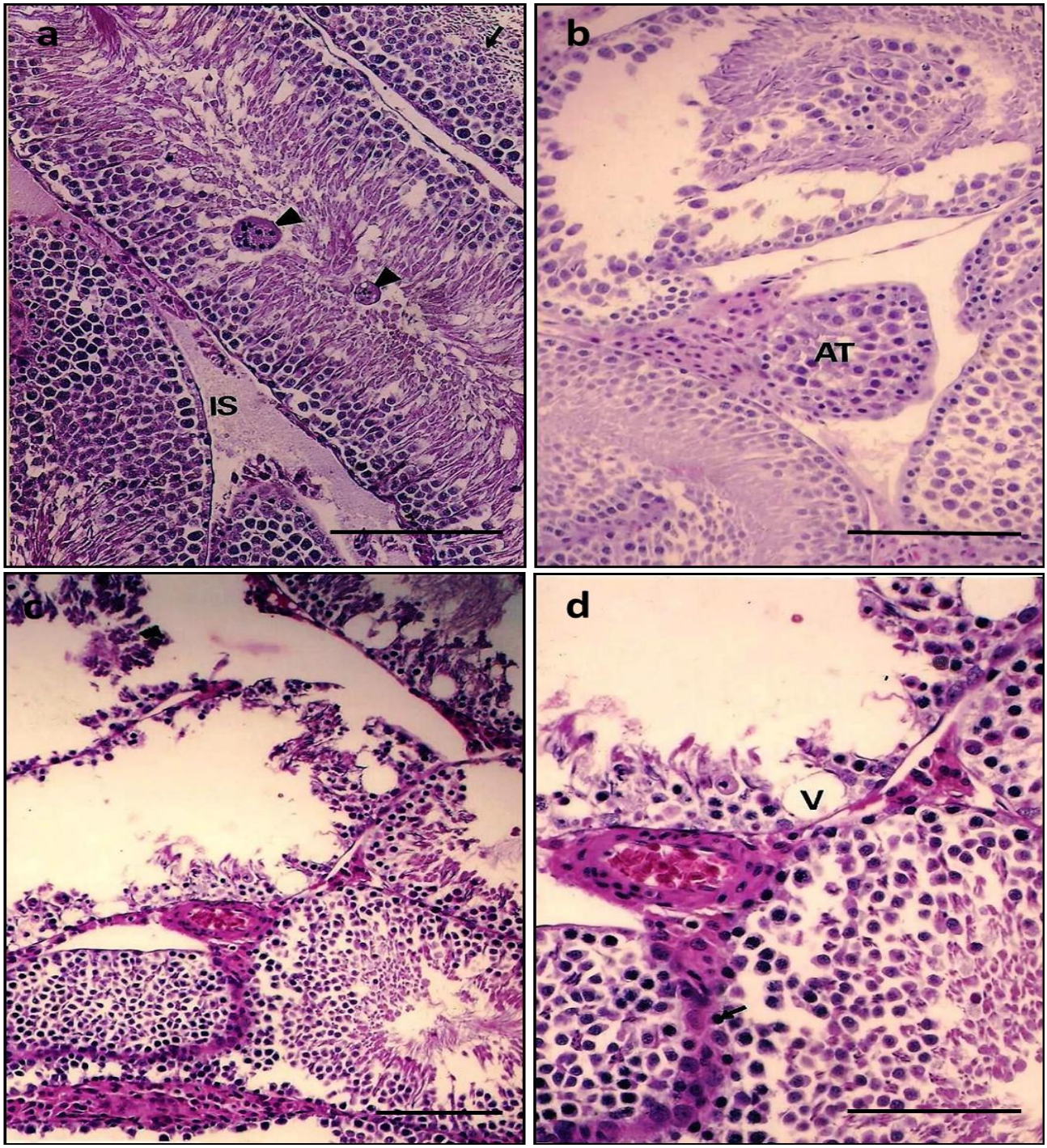


Plate (2a-d): L.M. Testicular cross sections of young rat group (G_2) treated with low dose of Fenitrothion (H. & E.).(a): Section of treated testis (G_{2a}) showing sloughing of germ cells into the tubular lumen (arrows); scale bar = 100µm.(b): Section of treated testis (G_{2b}) showing intratubular sloughed germ cells and slightly disturbed germinal epithelium. Note also lysis of fibrous connective tissue layer surrounding ST; Interstitial tissue cell (IT); scale bar = 100 µm.(c) Section of treated testis (G_{2c}) showing sloughed late spermatids (LSD) & intact spermatocyte. Note sertoli cells (SC) & vacuolated spermatogonia (arrow); scale bar = 20 µm. (d) : Section of treated testis (G_{2c}) showing reduction in germinal epithelium height; formation of multinucleated giant cells (MG) & proliferation of interstitial tissue (IT). Note nuclear damage of round spermatids (arrow) & proliferation of blood vessels (BV); scale bar = 100µm.

Plate (3a-d) : L.M. Testicular cross sections of young rate group (G_3) treated with high dose of Fenitrothion (H & E). (a) : Section of treated testis (G_{3a}) showing heterogeneity of germinal epithelial damage of seminiferous tubules. Note hypo-spermatogenesis (arrows), oedema (O) and germ cells lysis & disturbance in germinal epithelium in the seminiferous tubules. Note also lysis in fibrous layer surrounding tubules; scale bar = 100 μm.(b) : Section of treated testis (G_{3b}) showing general loss of germ cells (necrosis). Notice necrotic germ cells (GN) with pyknotic nuclei and highly eosinophilic cytoplasm; scale bar = 100 μm.(c) : Section of treated testis (G_{3c}) illustrating deformed and atrophied seminiferous tubules (AT); increased amount of interstitial tissue (IT) components; fibrosis and coagulation in blood vessel (BV) & absence of spermatozoa from lumen of nearly intact tubules; scale bar = 100 μm. (d) : High power from previous section. Note, no evidence of spermatogenesis is noted in atrophied tubules (AT). (aspermatogenesis); nearly intact sertoli cell nuclei (Sc) Note also strong inflammatory cell infiltration in interstitial cell (IT); scale bar = 20 μm.



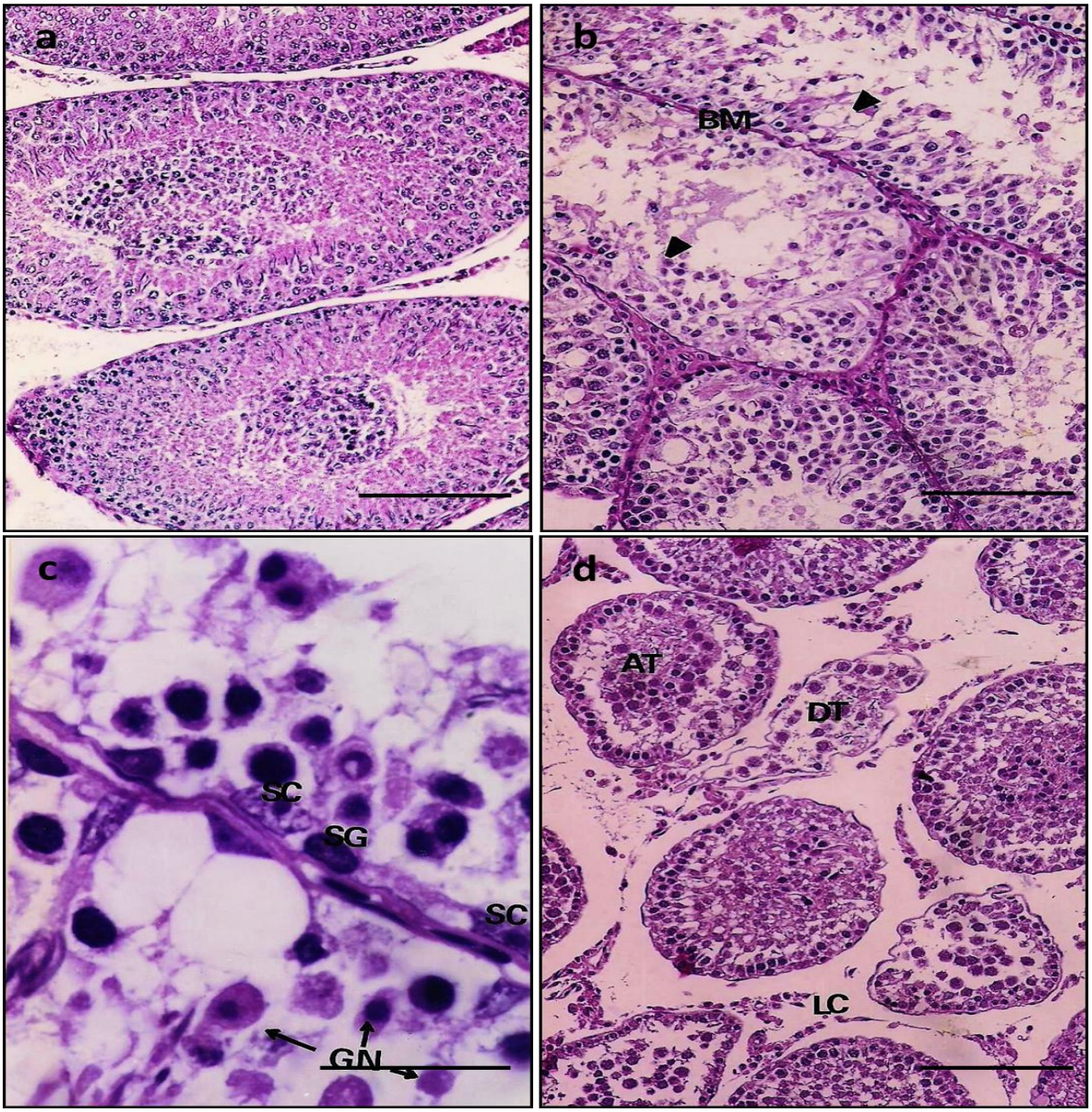


Plate (4a-d) : L.M. Testicular cross sections of adult rat group (G_2) treated with low dose of Fenitrothion (H & E).(a) : Section of treated testis (G_{2a}). Note tubules populated with all stages of spermatogenesis, but cellularity is relatively low and tubules appears dilated with low amount of mature spermatozoa. Note oedema in intertubular space (IS). multinucleated giant cells (head arrows) and residual bodies (arrow) in tubular lumen; scale bar = 100µm. (b) : Section of treated testis (G_{2b}) showing general loss of germ cells through desquamation and necrosis. Note atrophied (AT) and deformed tubules; scale bar = 100µm. (c) : Section of treated testis (G_{2c}). Note damaged seminiferous tubules & dilated tubules with disorganized epithelium and the generalized loss of germ cells; scale bar = 200 µm. (d) : High power from above section showing vacuolization (V) of tubular epithelium with massive spermatogenic cell necrosis. Note : loss of the normal germ cell associations; fibrosis and congestion of blood vessel; thickening of tubular basement membrane (arrow) scale bar = 100 µm.

Plate (5a-d) : L.M. Testicular cross sections from adult rat group (G_3) treated with high dose of Fenitrothion (H & E). (a) : Section of treated testis (G_{3a}) showing tubular lumen full -of desquamated germ cells but still have nearly normal stratified germinal epithelium; scale bar = 100 µm. (b) : Section of treated testis (G_{3b}) illustrating dramatic loss of germ cells through necrosis in some tubules

(head arrow), other tubules showed empty lumen free from spermatozoa & elongated spermatids. Note thick and intensely stained basement membrane (BM); scale bar = 100 μm. (c) : High power from previous section illustrating severe necrosis of germ cells (GN) associated with stop spermatogenesis. Note; outlines of deformed sertoli cell nuclei (SC) and few spermatogonia (SG) are apparent; scale bar = 20 μm. (d) : Section of treated testis (G_{3c}) showing atrophied tubules (AT) filled with cellular debris and deformed tubules (DT) with no evidence of spermatogenesis. Note damaged and necrotic Leydg's cells (LC); scale bar = 100 μm.

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