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EFFECT OF INHALATION OF PYRETHROID-BASED MOSQUITO REPELLENT ON THE SEMINIFEROUS TUBULES OF ADULT AND GROWING ALBINO RAT

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INTRODUCTION

Pyrethroid insecticides are synthetic analogues of the natural pyrethrins in flowers of the genus Chrysanthemum. They form, together with chlorinated hydrocarbons (DDT, dieldrin and lindane), organophosphorous compounds (Parathion, melathion, and diazinon) and methyl carbamate esters (Aldicarb, carbofuran and carbaryl), the four major classes of insecticides (**Casida et al., 1983**).

Based on the symptomatology after acute intoxication of the insects, pyrethroids fall into two classes: Type I, non-α-cyano-pyrethroids, such as permethrin, which affect generally the peripheral body activities and type II pyrethroids -characterized chemically by a cyano substituent-, such as cyhalothrin, which induce central actions (**Wouters and Van den Bercken, 1978; Lund and Narahashi, 1983; Narahashi, 1985**).

Pyrethroids are being used widely as agents of good insecticidal activity with low mammalian toxicity and rapid biodegradation. They are commonly used in mosquito repellents in the form of vapourizing mats, coils, scented sticks and liquidators (**Miyamoto et al., 1976**; **Warui, 1992; Beat et al., 1997**). Because these repellents are used routinely overnight, this may allow humans to inhale their vapors over a long period of time.

Although the synthetic pyrethroids are described as safe substances, their widespread use, their high nonselective potency and their considerable stability in the environment make them potentially toxic (**Casida et al., 1983**). Moreover, sustained contamination results from the adsorption of pyrethroids to small dust particles and various other surfaces.

Intoxication of mammals, including humans, by pyrethroids has been observed in many studies. Such cases included chromosomal aberrations (**Kandil and El Tarras, 1989**), inflammatory changes in the lungs and liver (**Ashmawy, 1992**), induction of insulin-dependent diabetes mellitus (**Tipton and Singer, 1993**), movement disorders (**Schulz and Beal, 1994**), Parkinson-like syndrome (**Müller-Mohnssen and Hahn, 1995**) and depression of fertility and reproductive power (**Hu et al., 2002; Mani et al., 2002; El-Demerdash et al., 2004**). **Cantalamessa (1993) and Gupta et al.**

(1999) reported a higher sensitivity of the neonate rats to the pyrethroid toxicity.

The spermatogenic tissue in the testis of man and animals was found to be affected on exposure to many types of insecticides **(Johnson et al., 2002; Mahgoub and El-Medany, 2001; Bustos-Obregon; Gonzalez-Hormazabal, 2003).** An attempt has been made in this work to investigate the effect of repeated inhalation of commercially available pyrethroid-based mosquito repellents by adult and growing rats on the histological and ultra-structural picture of the seminiferous tubules of albino rats. The study was extended further to see whether the changes that occurred following inhalation of pyrethroids were reversible following cessation of the exposure.

MATERIALS AND METHODS

Sprague-Dawley albino rats were used in this study including twenty adult male rats weighing 200-250 gm and 8 dams with their newly born pups (8 pups/dam, no selection of sex at this age). At the age of 3 weeks, the sex could be easily determined and 32 male growing rats were selected. The animals were divided into three groups as follow:

Goup I (control group): consisted of 4 adult rats and sixteen male growing rats. The control rats were housed in wire cages -the young pups together with their mothers- under standard environmental conditions and were given free food and water supply.

Group II (experimental group): consisted of 12 adult and 12 male growing rats. The adult and the young experimental rats were housed in wire cages (40x 25x 18cm) -the growing rats together with their mothers- in an isolated room. According to the design of **Gupta et al. (1999)**, a partition of Perpex sheet with numerous holes was provided in each cage. The experimental rats were kept on one side of this partition and on the other side one tablet of the commercially available mosquito mat "Ezalo" was left to fumigate in its special device. Each tablet contained 9.5 mg Bioallethrin 93% -a type I synthetic pyrethroid- and 14.4 mg Pyrethrum 95% -a natural pyrethrin-. The tablets were changed every day for 9 successive weeks. The "Ezalo" tablets were usually exhausted within 10 hours. The experimental rats were given free food and water supply. The dimensions of the experimental room were 3x2x3 m. Four adult and four growing experimental rats were killed 3, 6 and 9 weeks from the beginning of the experiment together with age matched control growing rats.

Group III (recovery group): consisted of 4 adult and 4 male growing rats. After exposure for 9 weeks to "Ezalo" vapor the rats were withdrawn from the experimental room to have a chance for recovery before being sacrificed together with those of the adult control rats and age matched growing control rats (13 weeks).

For all animals, sacrifice was performed by an overdose of ether.

A- Light microscopic study:

1-General histological examination:

Pieces from the testes of treated and control rats were fixed in neutral formalin and processed to paraffin blocks, sectioned at 5um thickness then stained with hematoxylin and eosin and examined under the light microscope (**Drury and Wallington, 1980**).

2-Morphometric study:

The diameters of the seminiferous tubules were measured by using Leica Quin-500 image analyzer computer system. In each animal specimen, transverse sections of the testes stained with hematoxylin and eosin were used to measure the two diagonal diameters of 100 seminiferous tubules, using 40x magnification. Two hundred readings were obtained for each specimen and the mean values were calculated. Student t test was performed to test for the significance of difference between the means of each experimental group as compared to the age matched control group. The data were collected and analyzed using the Statistical Package for the Social Sciences (SPSS) version 11 under windows XP.

B- Electron microscopic study:

Pieces of about 1mm from the testes of adult and growing rats exposed to pyrethroid vapors for 9 weeks as well as those of control adult rats were fixed in 4% glutraldehyde for 6 hours then transferred to phosphate buffered saline overnight. The specimens were post fixed in buffered 1% osmium tetra-oxide, dehydrated in ascending grades of alcohol and embedded in oraldite. Ultra-thin sections -60 to 100 nm thickness- were obtained, stained by uranyl acetate **(Watson, 1958)** and lead citrate **(Reynolds, 1963)** for examination by the transmission electron microscope.

RESULTS

Light microscopic study:

Under the light microscope, the seminiferous tubules of adult control rats were lined with spermatogenic epithelium consisting of several layers of spermatogenic cells at different stages of development: spermatogonia resting on the basement membrane together with the nursing (Sertoli) cells, primary spermatocytes –which were the largest cells-, early and late spermatids towards the lumina of the tubules. The intercellular spaces were occupied by cytoplasmic processes of Sertoli cells. The lumina contained

large numbers of spermatozoa. The tubules were packed together leaving narrow intertubular spaces (Figs. 1a, 1b).

Examination of the testes of adult rats exposed to inhalation of Pyrethroid vapor revealed attenuation of the spermatogenic epithelium which was progressive with increasing the duration of exposure. After 3 weeks, the seminiferous tubules were lined with high germinal epithelium formed of all types of spermatogenic cells which were slightly separated from each other. In some tubules, wide intercellular spaces appeared among the spermatogenic cells especially towards the basement membrane. The lumina of many tubules were free of spermatozoa and contained many residual bodies (Figs. 2a, 2b). With increasing the duration of exposure to 6 weeks, the tubules were lined with moderately high spermatogenic epithelium formed of all types of spermatogenic cells. Sites of radial fragmentation of the spermatogenic epithelium appeared frequently. Near the basal layer, empty spaces denoting rarefaction of the cytoplasm of the Sertoli cells were very common findings. The lumina contained very few spermatozoa and were filled with necrotic debris. Signs of shrinkage of the tubules in the form of relative decrease in diameter (Table I), irregularity of the basement membrane and widening of the intertubular spaces were observed (Figs. 3a, 3b). With further prolongation of exposure to 9 weeks, there was a marked thinning out of the tubular epithelium being formed mainly of Sertoli cells and spermatogonia with darkly stained nuclei resting on the basement membrane together with few degenerated spermatogenic cells. The lumina were filled with exfoliated degenerated cells with dark pyknotic nuclei and few spermatozoa. The tubules were separated by wide intertubular spaces. The basement membrane was festooned in many places (Figs. 4a, 4b). Statistically significant decrease in the mean diameter of the seminiferous tubules as compared to that of the adult control group was recorded (Table I) ($P<0.05$).

On the other hand, the spermatogenic epithelium lining the seminiferous tubules of growing rats exposed to Pyrethroid vapor was severely affected in all stages of the experiment as compared to those of age-matched control rats. At the age of 3 weeks, the tubular epithelium of the testes of control rats consisted of spermatogonia -together with Sertoli cells- and several layers of primary spermatocytes. The lumina were narrow and free of spermatocytes (Fig. 5a, 5b). In the experimental rats of the same age group, a low tubular epithelium leaving wide tubular lumina was observed. The epithelium was formed of Sertoli cells and spermatogonia with few primary spermatocytes with dark small nuclei. In many tubules the spermatogenic epithelium was separated from the basement membrane by a line of cleavage (Figs. 6a, 6b). At the age of 6 weeks, early spermatids appeared near the lumina in the tubules of control rats, though the lumina were still lacking spermatozoa (Figs. 7a, 7b). After 6 weeks of exposure, the germinal epithelium was thinned out, being formed of Sertoli cells, spermatogo-

nia and few primary spermatocytes with dark nuclei which were widely separated from each other. Spermatids were absent denoting maturation retardation of the germinal cells (Figs. 8a, 8b). The seminiferous tubules of the 9 weeks old control rats were lined with full thickness germinal epithelium with lumina filled with spermatozoa (Figs. 9a- 9b). The germinal epithelium of young rats exposed to Pyrethroid vapors for 9 weeks was severely attenuated. The basement membrane was lined by shrunken Sertoli cells, spermatogonia with dark nuclei and scanty degenerated spermatocytes.The lumina were empty or contained exfoliated necrotic debris without spermatozoa (Figs. 10a, 10b). The intertubular spaces were strikingly widened in both 6 weeks and 9 weeks old growing experimental animals (Figs. 8a, 10a). Moreover, statistical analysis of the tubular diameter of young rats of different groups revealed significant reduction in the mean values after 3, 6 and 9 weeks of exposure to Pyrethroid vapors (Table I) ($P<0.05$) as compared to those of the age-matched control rats.

In animals of both the adult and growing groups exposed to pyrethroid inhalation for 9 weeks and then allowed to recover for 4 weeks, remarkable improvement of the overall picture of the seminiferous tubules was observed. This improvement was manifested by restoration of the thickness of the spermatogenic epithelium and its normal architecture. Most of the tubules were lined by moderately high germinal epithelium showing all types of spermatogenic cells. Moreover, some tubules showed full thickness of the spermatogenic epithelium with large number of spermatozoa in the lumina. Also, the wide intertubular spaces observed in both adult and growing rats after exposure for 9 weeks had disappeared with a relative increase in the tubular diameter. However, recovery of the germinal epithelium was not complete as some tubules were lined with low epithelium and others showed areas of separation between the spermatogenic cells usually near the basement membrane. Also, the lumina of some tubules were free of spermatozoa (Figs.11a, 11b).

B. Electron microscopic study:

Three areas of the seminiferous tubules -the basal area, the ad-luminal area and the lumen- were examined. The basal compartment included cells resting on the basement membrane and consisted of the basal parts of the Sertoli cells and spermatogonia. The ad-luminal compartment consisted of spermatocytes and spermatids at different stages of development together with Sertoli cell processes. The lumen contained the end products of spermatogenesis; the spermatozoa.

Electron microscopic examination of testicular sections of control rats revealed the normal appearance of different spermatogenic cells. At the basal compartment, Sertoli cells had polymorphic nuclei with finely dispersed dusty chromatin and a characteristically prominent single nucleolus.

The nuclei often showed deep indentations. Their abundant cytoplasm had both lateral and apical processes that surrounded different spermatogenic cells coming in close contact with them and extending to the tubular lumen (figs. 12a, 12b). Their cytoplasm contained numerous thick walled slender or cup shaped mitochondria, few dense bodies, and few vacuoles (fig. 12a). The cell membranes of adjacent Sertoli cells often showed junctional complexes (fig. 12a) and their cytoplasmic processes frequently tightly interdigitated (fig. 12b). The spermatogonia were resting on the basement membrane. They were embraced by Sertoli cell processes and had oval nuclei with coarse granular chromatin which showed mild clumping around the periphery of the nucleus (fig. 12b). At the ad-luminal compartment, spermatocytes at different stages (fig. 12c) and early spermatids (with nuclear acrosomal granules or caps) (fig. 12d) were seen. Their cytoplasm contained many characteristically vacuolated peripherally situated mitochondria (figs. 12c, 12d). Late spermatids were recognized by their elongated highly dense nuclei (fig. 12e). Minimal intercellular spaces were observed; the cells were either in direct contact with each other or were surrounded by Sertoli cell processes (figs. 12b, 12c, 12d, 12e). In the lumen, cross sections in the heads, mid pieces or tails of spermatozoa were observed (fig. 12f).

The ultrastructural features of the basal compartment of seminiferous tubules of experimental rats of both adult and growing rats exposed to Pyrethroid vapor for the longest duration (9 weeks) revealed that Sertoli cells showed condensation of chromatin in the nucleoplasm especially near the nuclear membrane. Sertoli cell processes seemed to be retracted, losing contact with the neighboring spermatogenic cells leaving wide intercellular spaces especially near the basement membrane (figs. 13a, 14a, 14b). Sertoli-Sertoli cell junctions showed multiple gaps (fig. 13a). Their mitochondria showed the characteristic normal appearance (figs. 13a, 14a) except in few areas where Sertoli cell processes contained numerous vacuolated mitochondria with disrupted cristae (fig. 14c). In the spermatogonia, nuclei showed increased clumping of chromatin. Widening of the perinuclear space was observed in some cells. Wide spaces were present between spermatogonia denoting retraction of Sertoli cell lateral processes (fig. 14b). In the ad-luminal compartment of seminiferous tubules of rats of these groups, many spermatogenic cells showed signs of severe nuclear damage in the form of condensed chromatin (fig. 13b, 13c) and irregular or interrupted nuclear envelope (fig. 13c). In some cells, the nucleus was totally occupied by highly clumped chromatin material (fig. 14c), and in others rarefaction of chromatin was observed (figs. 14d). The cells were surrounded by highly vacuolated Sertoli cell processes (figs. 13b, 14c) and wide intercellular spaces were noted (fig. 14d). In the lumen, many spermatocytes (fig. 14e) and spermatids (fig. 13d) which sometimes appeared normal in morphology were prematurely exfoliated. Occasionally, abnormally formed spermatozoa were encountered in the adult group (fig. 13e).The

lumen was filled with severely vacuolated residual bodies of degenerated cells of abnormal architecture (fig. 14e). These changes were observed in the growing as well as in the adult groups. However, they were more pronounced in the growing rats in which cells at late stages of spermatogenesis (elongated spermatids and spermatozoa) were rarely encountered.

Table (I): Measurements of the diameters of the seminiferous tubules of the control and experimental adult rats.

Group	Number of rats	Mean (in mm)	SD	P Value
Control	4	311.9	± 56.3	
3 weeks exposure	4	320.3	± 49.5	
6 weeks exposure	4	275.4	± 33.7	
9 weeks exposure	4	235.5	± 26.8	*
9 weeks exposure + 4 weeks recovery	4	287.2	± 39	

- SD = Standard deviation.
- Student t test:
* Significant with respect to the control group at $P < 0.05$.

Table (II): Measurements of the diameters of the seminiferous tubules of the control and experimental growing adult rats.

Group	Number of rats	Mean (in mm)	SD	P Value
3 weeks exposure	4	89.1	± 20.4	*
Age matched control	4	147.4	± 32.2	
6 weeks exposure	4	123.8	± 21.1	*
Age matched control	4	185.2	± 34.7	
9 weeks exposure	4	182.3	± 18.3	*
Age matched control	4	238.1	± 46	
9 Weeks exposure + 4 Weeks recovery	4	240.8	± 42.7	
Age matched control	4	262	± 37.9	

- SD = Standard deviation.
- Student t test:
* Significant with respect to the age matched control group at $P < 0.05$.

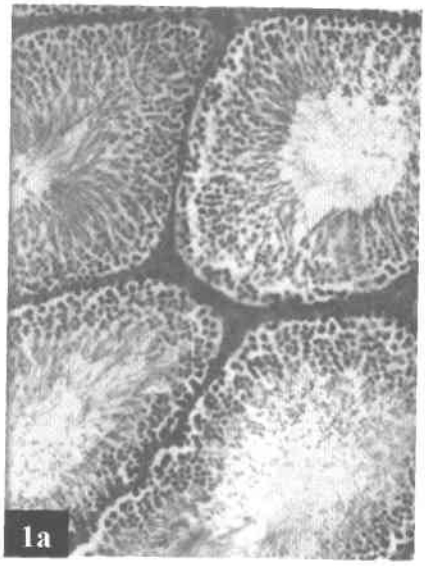

Fig. (1-a): A photomicrograh of a transverse section of the testis of an adult control rat. The seminiferous tubules are lined with several layers of spermatogenic cells. The intertubular spaces are narrow. **(H&E; X100)**

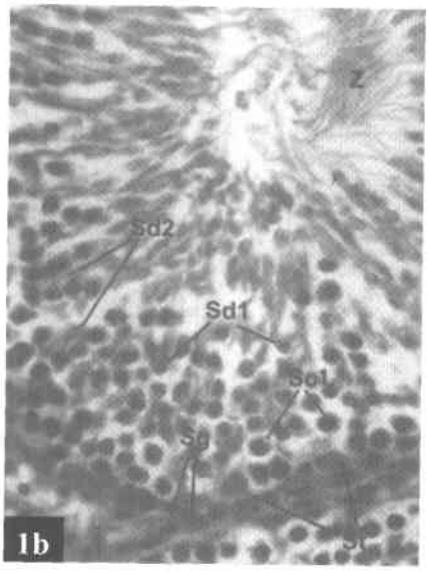

Fig. (1-b): A higher magnification of the previous section showing the seminiferous epithelium which is formed of spermatogonia (Sg), primary spermatocytes (Sc1), early and late spermatids (Sd1, Sd2).The lumen is full of spermatozoa (Z). Many Sertoli cells (St) are also seen resting on the basement membrane. **(H&E; X400)**

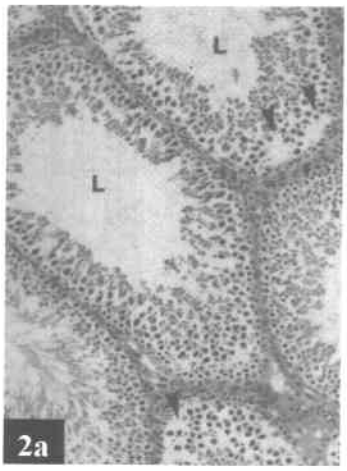

Fig. (2-a): A photomicrograh of a transverse section of the testis of an adult rat exposed to pyrethroid vapor for 3 weeks. The seminiferous tubules are lined with high spermatogenic epithelium many having sperm free lumina (L). The germ cells are slightly separated from each other. Some wide intercellular spaces appear among the germ cells (arrow heads). **(H&E; X100)**

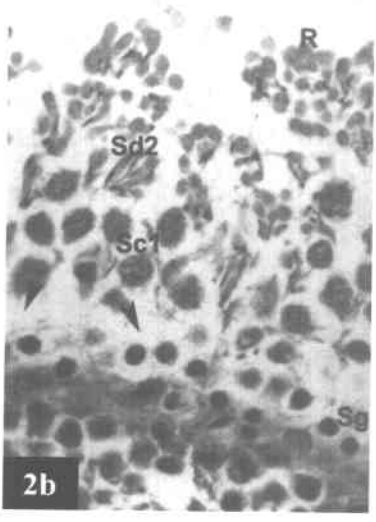

Fig. (2-b): A higher magnification of the previous section showing the seminiferous epithelium which is formed of spermatogonia (Sg), primary spermatocytes (Sc1) and late spermatids (Sd2). A space is observed between the spermatogonia and primary spermatocytes (arrow heads).Many residual bodies are seen towards the lumen (R). **(H&E; X400)**

Fig. (3-a): A photomicrograh of a transverse section of the testis of an adult rat exposed to pyrethroid vapor for 6 weeks. The seminiferous tubules are lined with moderately high spermatogenic epithelium. Sites of radial fragmentation (RF) appear among the epithelial cells. The lumina contain necrotic debris (N). Note the wide intertubular spaces. **(H&E; X100)**

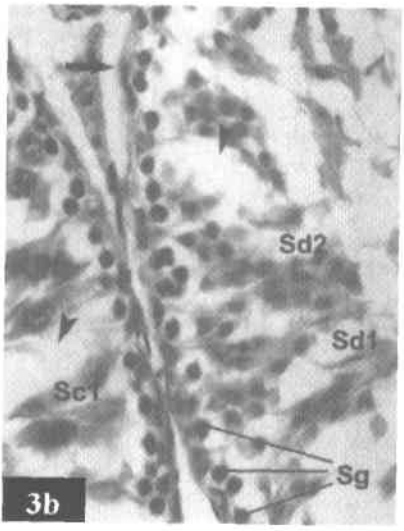

Fig. (3-b): A higher magnification of the previous section showing the seminiferous epithelium which is formed of spermatogonia (Sg), primary spermatocytes (Sc1), early and late spermatids (Sd1, Sd2).Many empty spaces are seen near the basal layer (arrow heads). Irregularity of the basement membrane is observed (arrow). **(H&E; X400)**

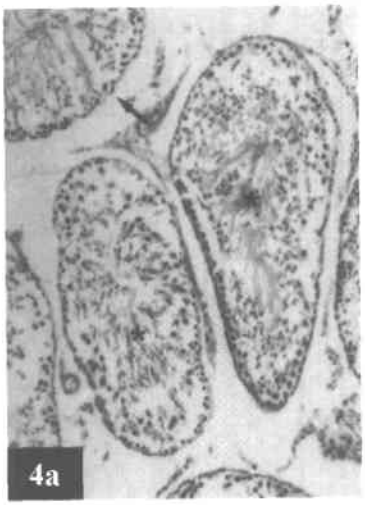

Fig. (4-a): A photomicrograh of a transverse section of the testis of an adult rat exposed to pyrethroid vapor for 9 weeks. The seminiferous tubules are lined with low degenerated spermatogenic epithelium. The lumina contain few spermatozoa intermingled with sloughed necrotic cells (asterisks). Note the festooning of the basement membrane (arrow) of some tubules and the wide intertubular spaces. **(H&E; X100)**

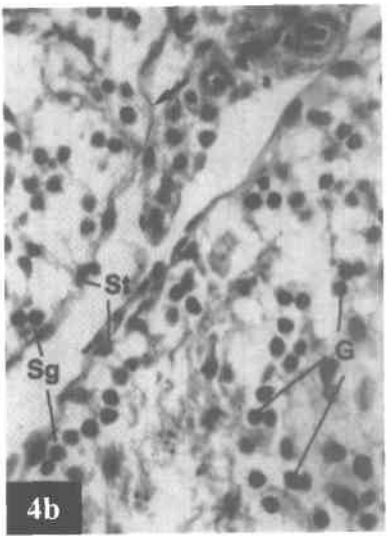

Fig (4-b): A higher magnification of the previous section showing germinal cells (G) with dark nuclei mostly exfoliated into the lumen. On the basement membrane, Sertoli cells (St) and spermatogonia (Sg) with darkly stained nuclei are seen. Festooning of the basement membrane (arrow) is observed. **(H&E; X400)**

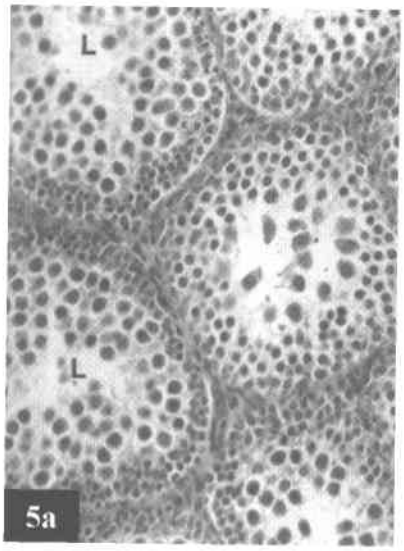

Fig. (5-a): A photomicrograh of a transverse section of the testis of a 3-weeks old control rat showing seminiferous tubules lined with high tubular spermatogenic epithelium. The lumina (L) are narrow and free of spermatozoa. **(H&E; X200).**

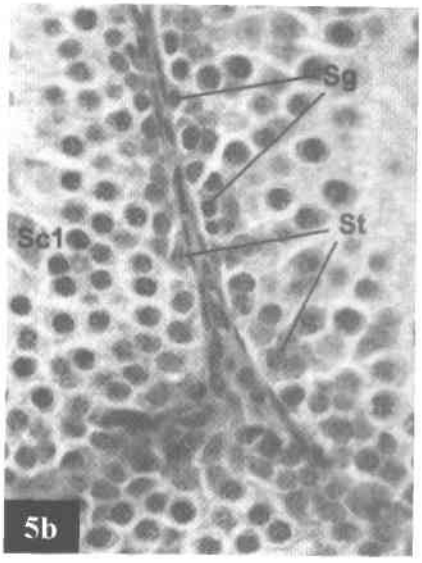

Fig. (5-b): A higher magnification of the previous section showing the seminiferous epithelium which is formed of spermatogonia (Sg), primary spermatocytes (Sc1) together with Sertoli cells (St) near the basement membrane. **(H&E; X400)**

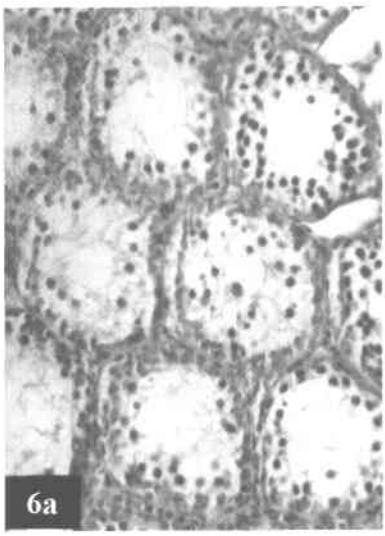

Fig. (6-a): A photomicrograh of a transverse section of the testis of a 3-weeks old rat exposed to pyrethroid vapor since birth most of the tubules show low spermatogenic epithelium with widening of the tubular lumen. Note the smaller diameter of the seminiferous tubules when compared to those of the control rat of the same age (Fig.5-a). **(H&E; X200)**

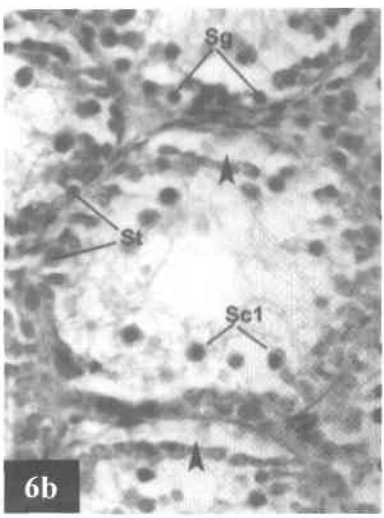

Fig. (6-b): A higher magnification of the previous section showing the seminiferous epithelium which is formed of Sertoli cells (St), spermatogonia (Sg) and few primary spermatocytes (Sc1) with deeply basophilic nuclei. In many places, the spermatogenic epithelium is separated from the basement membrane (arrow heads). **(H&E; X400)**

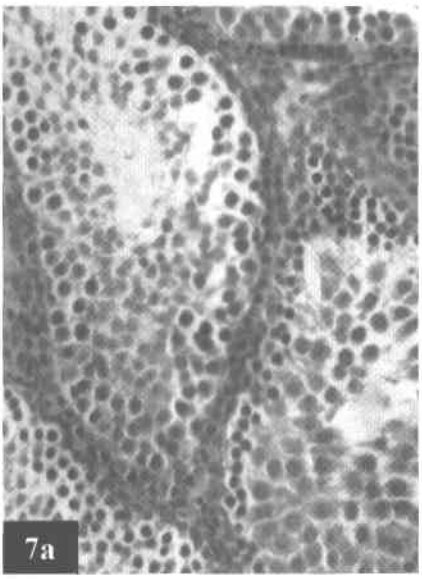

Fig. (7-a): A photomicrograh of a transverse section of the testis of a 6-weeks old control rat showing high tubular epithelium with narrow sperm free lumina. **(H&E; X200)**

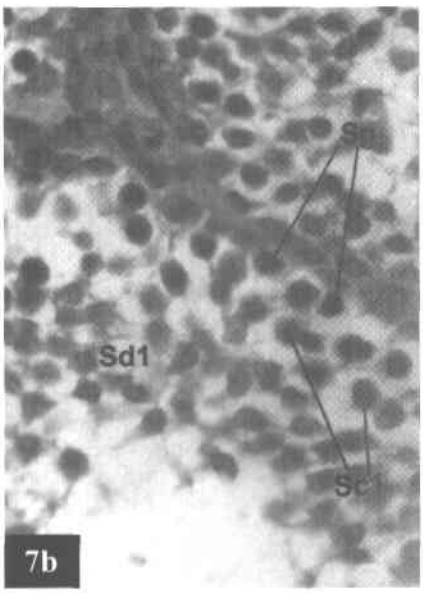

Fig. (7-b): A higher magnification of the previous section showing the seminiferous epithelium which is formed of spermatogonia (Sg) and primary spermatocytes (Sc1).Early spermatids (Sd1) appear in many tubules near the lumen. **(H&E; X400)**

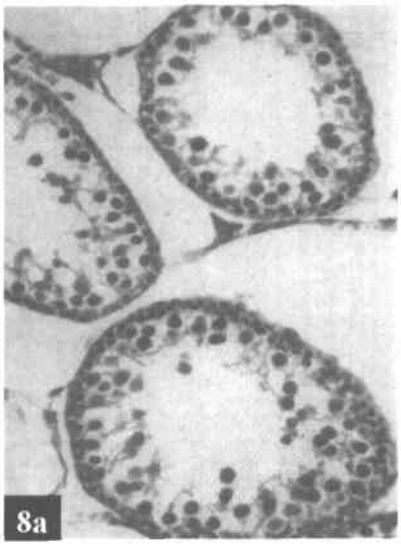

Fig. (8-a): A photomicrograh of a transverse section of the testis of a 6-weeks old rat exposed to pyrethroid vapor since birth. The seminiferous tubules are lined with low spermatogenic epithelium. Note the small diameter of the tubules as compared to those of the control rat of the same age (Fig. 7-a) and the wide intertubular spaces. Sertoli cell nuclei (St) are seen near the basement membrane. **(H&E; X200)**

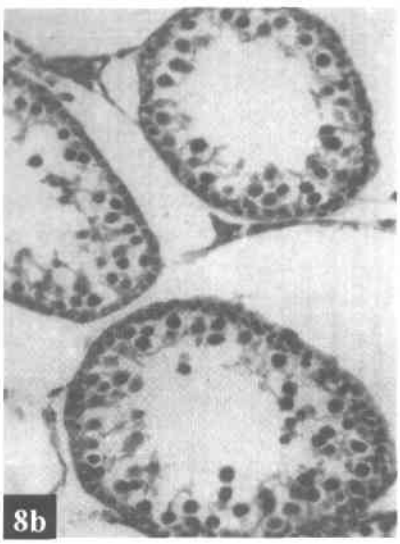

Fig. (8-b): A higher magnification of the previous section showing the seminiferous epithelium which is formed of formed of spermatogonia (Sg), few primary spermatocytes (Sc1) with darkly stained nuclei. **(H&E; X400)**

Fig. (9-a): A photomicrograh of a transverse section of the testis of a 9-weeks old control rat. The seminiferous tubules show full thickness of the spermatogenic epithelium. Most of the lumina are full of spermatozoa (Z). **(H&E; X100)**

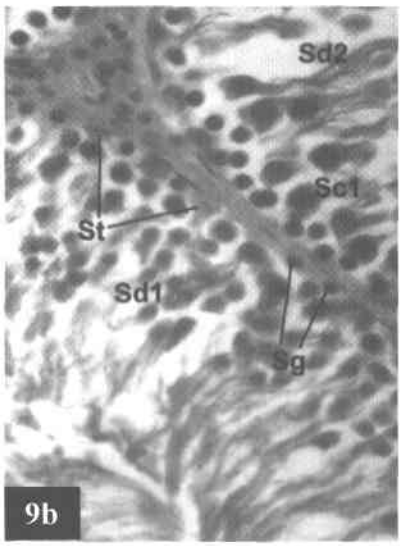

Fig. (9-b): A higher magnification of the previous section showing the seminiferous epithelium which consists of spermatogonia (Sg), primary spermatocytes (Sc1), early and late spermatids (Sd1, Sd2). Many Sertoli cell nuclei (St) are also seen resting on the basement membrane. **(H&E; X400)**

Fig. (10-a): A photomicrograh of a transverse section of the testis of a 9-weeks old rat exposed to pyrethroid vapor since birth. The seminiferous tubules are lined with low germinal epithelium. The lumina are empty or contain necrotic debris (asterisks). Note the wide intertubular spaces. **(H&E; X100)**

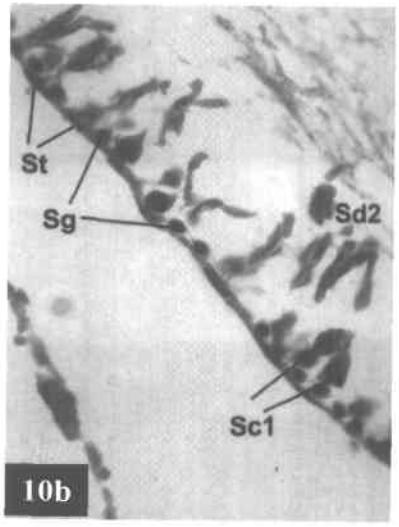

Fig. (10-b): A higher magnification of the previous section showing the seminiferous epithelium which consists of few spermatogonia (Sg) and primary spermatocytes (Sc1) with dark nuclei and deeply acidophilic cytoplasm together with exfoliated late spermatids (Sd2). Sertoli cells (St) with dark nuclei are seen resting on the basement membrane. **(H&E; X400)**

Fig. (11-a): A photomicrograh of a transverse section of the testis of a 13-weeks old growing rat exposed to pyrethroid vapor for 9 weeks and allowed to recover for 4 weeks. Many seminiferous tubules are lined with full thickness spermatogenic epithelium having lumina full of spermatozoa (Z). Other tubules are lined with moderately high epithelium with areas of separation between the cells (arrow heads) and sperm free lumina (L). Note the narrow intertubular spaces. **(H&E; X100)**

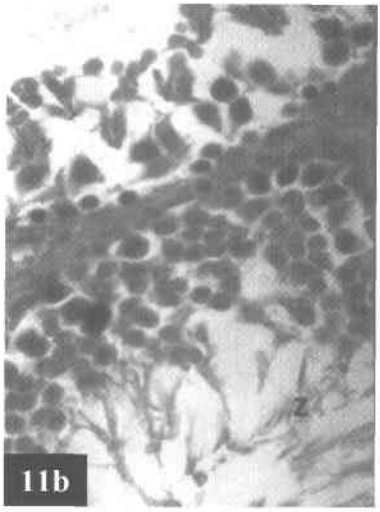

Fig. (11-b): A higher magnification of the previous section showing the adjacent sides of 2 seminiferous tubules. The lower tubule is lined with a full thickness epithelium with many spermatozoa (Z) while the upper one is lined with a low epithelium with no spermatozoa. **(H&E; X400)**

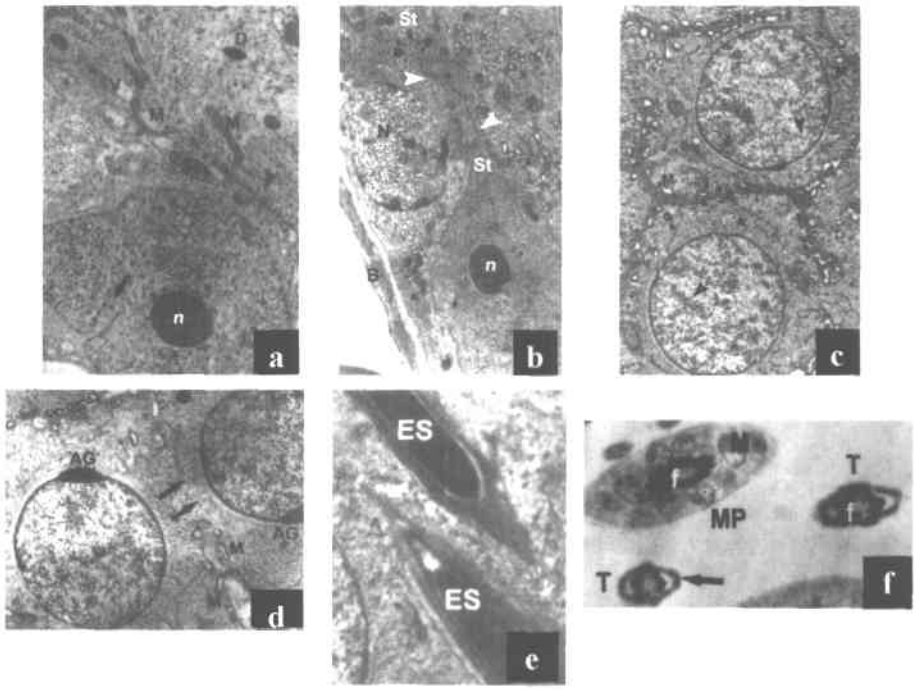

Fig. (12): Electron micrographs of the seminiferous tubules of adult rats of the control group showing:

a) The basal portion of a Sertoli cell. The nucleus has a deep indentation (arrow) and a single prominent nucleolus (n). The nucleus is occupied by finely granular chromatin. The abundant cytoplasm contains many thick walled slender or cup shaped mitochondria (M), few dense bodies (D) and few vacuoles (V). A tight junctional complex is seen between adjacent Sertoli cells (arrow head). **(x 3500)**

b) The basement membrane (B) on which rest a spermatogonium (top) and a Sertoli cell (bottom). The spermatogonium is surrounded by Sertoli cell processes (St) which tightly interdigitate (arrow heads). The chromatin content of its nucleus (N) is coarsely granular and shows peripheral clumping. The Sertoli cell nucleus has a characteristic prominent nucleolus (n) and fine granular chromatin. **(x 2500)**

c) Two primary spermatocytes at the pachytene stage with rounded nuclei containing granular chromatin in which synaptonemal complexes are formed (arrow heads). In the cytoplasm, vacuolated mitochondria (M) are situated near the cell membrane. No intercellular spaces are observed. **(x 5000)**

d) Two early spermatids with nuclear acrosomal granules (AG). The cytoplasm contains vacuolated peripherally situated mitochondria (M). The cells are in close contact. Note the cytoplasmic bridge connecting the 2 homologous cells (arrows). **(x 5000)**

e) Two elongated spermatids (ES) characterized by their elongated highly dense nuclei. No intercellular spaces are observed. **(x 5000)**

f) Cross sections in mid piece (MP) and tails (T) of spermatozoa free in the lumen. In the mid piece, mitochondria (M) are seen surrounding the axial filament (f) while in the tail piece, the axial filament is surrounded by a fibrous sheath (arrow). **(x 7500)**

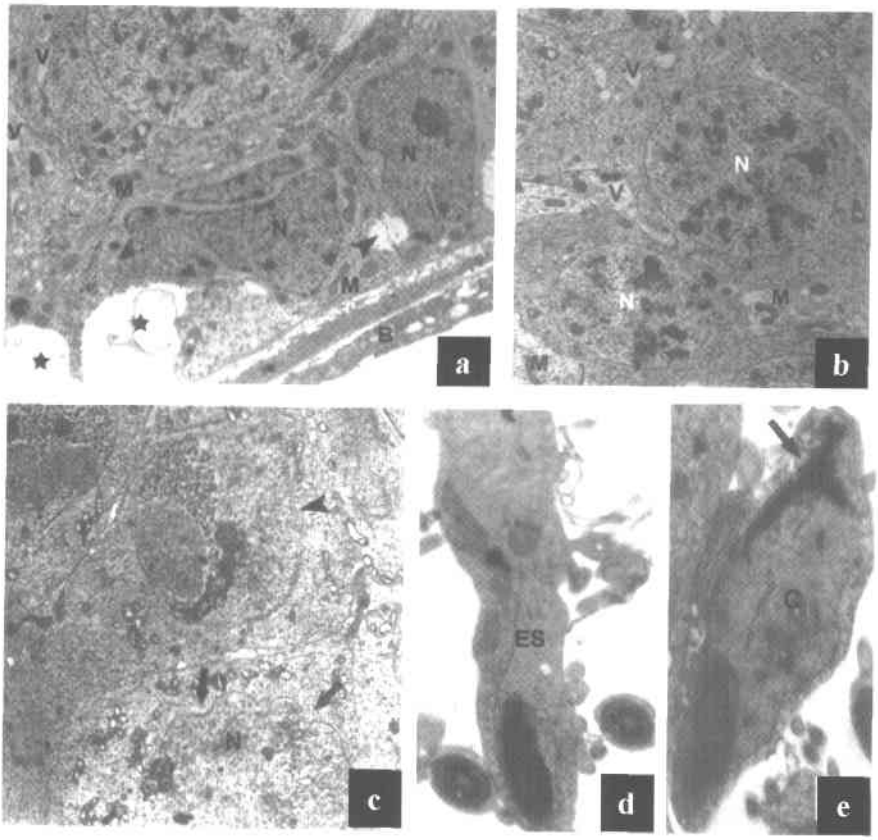

Fig. (13): Electron micrographs of the seminiferous tubules of adult rats exposed to pyrethroid vapor for 9 weeks showing:

a) Two Sertoli cells resting on the basement membrane (B). Their nuclei (N) show peripheral clumping of chromatin .Their cytoplasm contains several normal mitochondria (M) and many vacuoles (V). A gap (arrow head) is seen interrupting the intersertoli cell junction. Wide spaces (stars) appear near the basement membrane. **(x 2500)**

b) Primary spermatocytes at the pachytene stage. Clumping of chromatin material is observed in their nuclei (N). Sertoli cell processes surrounding the cells contain many vacuoles (V) and normal mitochondria (M). **(x 2500)**

c) A primary spermatocytes at the pachytene stage. The nucleus (N) has irregular nuclear membrane (arrows). An adjacent spermatogenic cell has a degenerating nucleus with clumped chromatin and interrupted nuclear membrane (arrow head). **(x 2500)**

d) An elongated spermatid (ES) prematurely exfoliated into the lumen. **(x 2500)**

e) An abnormally formed spermatozoon into the lumen. It has a bifid tail (arrow) and it retains a large amount of its cytoplasm (C). **(x 2500)**

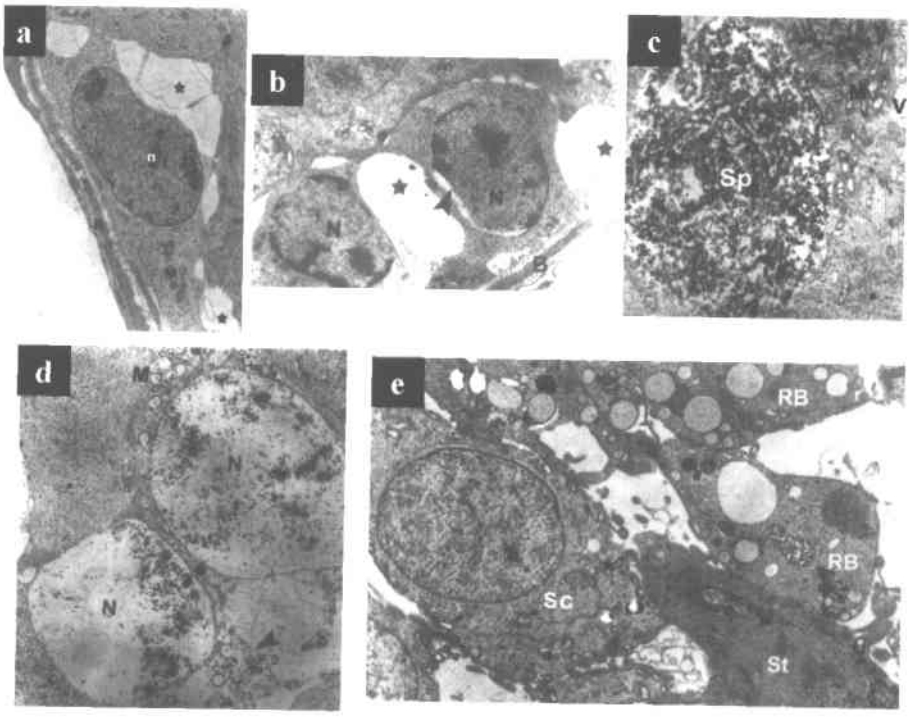

Fig. (14): Electron micrographs of the seminiferous tubules of growing rats exposed to pyrethroid vapor for 9 weeks showing:

a) A Sertoli cell resting on the basement membrane (B). Its nucleus has a small nucleolus (n) and exhibits clumping of chromatin material. Its cytoplasm contains normal mitochondria (M) but its processes lost contact with adjacent cells leaving wide intercellular spaces (stars). **(x 2500)**

b) Two spermatogonia resting on the basement membrane (B). Their nuclei (N) contain clumped chromatin. A perinuclear space (arrow head) is seen in one cell. Wide intercellular spaces are observed (stars). **(x 2500)**

c) A degenerated spermatogenic cell (Sp) totally occupied by clumped chromatin. The surrounding Sertoli cell process contains many mitochondria with disrupted cristae (M) and many vacuoles (V). **(x 5000)**

d) Two spermatogenic cells with degenerated nuclei (N) containing few clumps of chromatin. Their cytoplasm contains many ballooned mitochondria (M). At one side, the cell membrane is interrupted with pooling out of the cytoplasmic contents into the wide intercellular space (arrow head). **(x 2500)**

e) A primary spermatocyte with apparently normal structure (Sc) exfoliated into the lumen. A Sertoli cell process (St) is seen losing contact with the spermatocyte. Many highly vacuolated residual bodies (RB) are seen. **(x 2500)**

DISCUSSION

Pyrethroid-containing mosquito repellents are being widely used especially indoors and are usually left to vaporize overnight. Repeated use of these repellents results in chronic inhalation of their vapors. Many authors reported alteration of semen quality of adult male rats exposed to repeated doses of different types of pyrethroids in the form of decreased sperm count and sperm motility (%) with increased dead and abnormal sperms (**Hu et al., 2002**; **Yousef et al., 2003**; **El-Demerdash et al., 2004**). Also, **Elbetieha et al. (2001)** observed reduction in the reproductive power and fertility of adult male rats exposed to cypermethrin for 12 weeks when left to mate with unexposed females.

In this study, histological examination of the testes of adult rats exposed to pyrethroid vapor revealed alteration of the spermatogenic epithelium which ran parallel with the duration of the experiment, i.e. the longer the exposure, the more extensive the damage was. Alteration of the epithelium was in the form of decrease in the thickness of the spermatogenic epithelium with radial or focal fragmentation and abundance of degenerated forms. The lumina contained few spermatozoa with many necrotic bodies. Similar observations were reported by **El Gohary et al. (1999) and Elbetieha et al. (2001)**. A statistically significant decrease of the diameter of the seminiferous tubules of adult rats exposed to pyrethroid vapors for 9 weeks as compared to those of control rats was observed in this study.

As pyrethroid-based fumigants are currently used to protect neonates and children from mosquito bites, this study was extended to assess the sensitivity of the testicular germinal epithelium of neonatal rats to chronic pyrethroid intoxication in comparison to that of adult rats. The results demonstrated higher level of sensitivity of the neonate rats as their spermatogenic epithelium was severely affected even with the shortest period of exposure (3 weeks). Statistical study demonstrated significant reduction in the diameter of the seminiferous tubules of the growing experimental rats as compared to those of the age-matched controls in all stages of the experiment. It was suggested that pyrethroids –on prolonged exposure during the post natal period-caused a delay in testicular development and maturation. These findings are in agreement with those of **Cantalamessa (1993) and Gupta et al. (1999)** who confirmed that the sensitivity of neonatal rats to pyrethroid toxicity on different organs was higher, the younger the animals. **Cantalamessa (1993)** emphasized that this was probably due to incomplete development of the enzymes which catalyze the metabolism of pyrethroids in the liver of young animals. On the contrary, **Sheets (2000)** concluded that young animals were not more sensitive to sublethal doses of pyrethroid insecticide. This controversy might be explained by the fact that the latter author based his study on one acute dose of the insecticide.

Ultrastructurally, the toxic effect of pyrethroid vapor inhalation for 9 weeks on adult and growing rats involved all types of spermatogenic cells. This toxic effect was extensive in the spermatocytes, spermatids and spermatozoa –nuclear changes being more evident than cytoplasmic changes- up to the level of inducing necrosis. On the other hand, spermatogonia and Sertoli cells were less affected. The spermatogonia exhibited increased clumps of chromatin in the nucleus and occasional widening of perinuclear space. In the Sertoli cells, the nuclear changes were restricted to mild peripheral chromatin condensation. The main ultrastructural changes in Sertoli cells were observed in their cytoplasmic processes causing their retraction and loss of intimate contact with spermatogenic cells and loosening of Sertoli-Sertoli cell junctions particularly at the basal compartment. Sertoli cells exhibited an increased amount of vacuoles in their cytoplasm. However, mitochondria appeared normal except in very few sites. The main function of Sertoli cells of support and nutrition of spermatogenic epithelium seemed to be arrested. As these cells are the functional unit of the blood-testis barrier, retraction of their processes provided a gate-way for the toxin to reach the normally protected spermatogenic cells behind this guardian barrier. Sertoli cell dysfunction could account for the severe affection of spermatogenic cells and the abundance of prematurely exfoliated cells into the lumen. Such picture is in accordance with the concept of **Haschek and Rousseau (1991)** that Sertoli cells and spermatogonia are remarkably resistant to injury. These authors mentioned that most toxins lead to generalized germ cell depletion often leaving only Sertoli cells and spermatogonia within the tubules and this was referred to as maturation arrest.

Few studies reported recovery from harmful effect of pyrethroid intoxication on blood-brain barrier, liver and kidney **(Gupta et al., 1999)** and on erythrocytes **(Kale et al., 1999)**, but none have been found in the available literature concerning the possibility of recovery of the testicular epithelium. On the other hand, **Haschek and Rousseau (1991)** confirmed that following germ cell damage due to testicular toxicants, the chances of regeneration of the entire germ cell population and recovery of the functional spermatogenesis were good. They attributed this observation to the relative resistance of spermatogonia as well as the Sertoli cells to injury. Such postulation goes in parallel with the results of the present study: after withdrawal of exposure for four weeks, the spermatogenic epithelium in both adult and growing rats had almost completely recovered except for minimal affection in some seminiferous tubules. The sublethal affection of spermatogonia and Sertoli cells observed under the electron microscope in exposed groups might account for the regeneration of germinal epithelium: the former being the stem cell for other spermatogenic cells and the latter playing a vital role in spermatogenesis.

Many mechanisms have been postulated to be on the basis of pyrethroid induced toxicity. **Gupta et al. (1999) and Kale et al. (1999)** reported

that pyrethroids produced oxidative stress in intoxicated rats. Moreover, **El-Gohary et al. (1999), El-Demerdash et al. (2003), Yousef et al. (2003) and El-Demerdash et al. (2004)** confirmed the protective effect of different antioxidants against harmful effect of pyrethroids on reproductive parameters of male rats. Also, some pyrethroids were reported to be endocrine disruptors, causing a decrease in plasma testosterone **(Hu et al.,2002; Mani et al., 2002). Mani et al. (2002)** observed a decrease in marker testicular enzymes for testosterone biosynthesis and attributed fenvalarate induced male reproductive toxicity of rats to neuro-endocrine mediated phenomenon. However, **Kunimatsu et al. (2002) and Yamada et al. (2003)** denied anti-androgenic or estrogenic effect of different pyrethroids. On the other hand, interference with the mitochondrial respiratory chain and decrease in the activity of mitochondrial respiratory enzymes has been claimed by **Beat et al. (1997)** to be responsible for pyrethroid toxicity.

It thus appears from this study that repeated inhalation of pyrethroid–based mosquito repellents exerts toxic effects on the spermatogenic epithelium in the testis that is non–persistent in nature and could recover few weeks after cessation of exposure. It was also concluded that neonates are more sensitive than adults to the toxic effect of pyrethroids. Accordingly it is advised to avoid long term exposure to the pyrethroid–containing mosquito repellents especially for younger individuals.

SUMMARY

Pyrethroids are widely used natural and synthetic insecticides. Pyrethroid based vaporizing mats are being currently used as mosquito repellents especially for neonates and children. This study was planned out to investigate the possible hazardous effect of repeated inhalation of pyrethroid vapors on the epithelium of the seminiferous tubules of adult as well as growing male albino rats. The possibility of recovery of the seminiferous epithelium following withdrawal of exposure was also estimated.

Twenty adult and 32 new born male albino rats were divided to 3 groups each consisting of both adult and growing rats; group I: control group; group II: pyrethroid exposed group and group III: recovery group. Groups II were exposed to inhalation of the vapor of one Ezalo tablet per day until the day of sacrifice. Each tablet contained 9.5 mg Bioallethrin 93% –a synthetic pyrethroid- and 14.4 mg Pyrethrum 95% –a natural pyrethrin- and was exhausted after 10 hours. Animals of the group II were sacrificed after 3, 6 and 9 weeks from the beginning of the experiment together with age matched control growing rats. Animals of the group III were exposed to inhalation of Ezalo vapor for 9 weeks and then exposure was stopped 4 weeks before being sacrificed with adult control rats and age matched growing control rats.

Samples from the testes of adult and growing rats of different groups were examined under the light microscope (using hematoxylin and eosin stain). The diameters of the seminiferous tubules were measured using the image analyzer computer system and statistical analysis was performed to compare the collected data. Transmission electron microscopic examination was performed for samples of the testes of the rats of the adult control group, and of adult and growing rats of the second group exposed to pyrethroid vapor for the longest duration (9 weeks).

The results of this study demonstrated that repeated inhalation of pyrethroid vapors by both adult and growing rats caused remarkable attenuation of the seminiferous epithelium which was progressive with increasing the duration of exposure. All types of cells in the seminiferous tubules showed degenerative changes; spermatogonia and Sertoli cells were found to be the most resistant cells to the effect of the toxin. Growing rats were proved to be more sensitive to the toxic effect of pyrethroid vapors than adult rats. However, after cessation of exposure for 4 weeks, the seminiferous epithelium restored its normal architecture in most of the tubules in both adult and growing groups.

Accordingly it is advised to avoid long term exposure to the pyrethroid–containing mosquito repellents especially for younger individuals.

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الملخص العربى

تأثير استنشاق طارد الناموس المحتوي علي البيريثرويد علي القنيات المنوية للفئران البيضاء البالغة والنامية

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تعتبر البيريثرويدز من المبيدات الحشرية الواسعة الانتشار و تستعمل أقراص الناموس ايزالو التي تحتوي علي البيريثرويدز لطرد الناموس خاصة بالنسبة لحديثي الولادة والأطفال . وقد تم التخطيط لهذا البحث لدراسة الاثار الضارة المحتملة لاستنشاق ابخرة ايزالو بشكل متكرر علي الخلايا المبطنة للقنيات المنوية في كل من الذكور البالغة والذكور النامية في الفئران البيضاء. وقد تم أيضا دراسة احتمال التعافي بعد توقيف التعرض لأبخرة ايزالو.

استخدم في هذه التجربة عشرون من ذكور الفئران البيضاء البالغة و أثنان وثلاثون من ذكور الفئران البيضاء حديثة الولادة قسمت الي ثلاث مجموعات تحتوي كل منها على فئران بالغة وفئران نامية ؛ المجموعة الأولي: المجموعة الضابطة، المجموعة الثانية:المجموعة التي تعرضت لأبخرة البيريثرويدز، المجموعة الثالثة: مجموعة التعافي. تم تعريض فئران المجموعة الثانية لأبخرة قرص واحد من ايزالو يوميا حتي يوم التضحية. كل قرص من ايزالو يحتوي علي ٥،٩ مجم بيوأللثرين ٩٣% كبيريثرويد صناعي و ٤،١٤ مجم بيريثرم ٩٥% كبيريثرويد طبيعي، وكان القرص ينضب بعد ١٠ ساعات. تمت التضحية بفئران المجموعة الثانية بعد ٣ و ٦ و ٩ أسابيع من بدء التعرض مع الفئران النامية من نفس العمر من المجموعة الضابطة. أما المجموعة الثالثة فقد تم تعريضها لأبخرة الايزالو لمدة ٩ أسابيع ثم تم توقيف التعرض لمدة ٤ أسابيع أخري قبل التضحية بها مع الفئران البالغة والفئران النامية من نفس العمر من المجموعة الضابطة .

تم فحص عينات من خصية الفئران من المجموعات المختلفة بالمجهر الضوئي (بأستخدام صبغة الهيماتوكسيلين والأيوسين. و تم قياس أقطار القنيات المنوية بأستخدام محلل الصور و أجريت دراسة احصائية للمقارنة بين نتائج المجموعات المختلفة . تم أيضا فحص عينات الخصية من الفئران البالغة في المجموعة الضابطة ومن الفئران البالغة والنامية في المجموعة الثانية التي تعرضت لأبخرة الأيزالو لاطول فترة (٩ أسابيع) بالمجهر الألكتروني .

وقد أظهرت نتائج هذه التجربة أن الاستنشاق المتكرر لأبخرة ايزالو لكل من الفئران البالغة والفئران النامية يسبب تأثيرا سلبيا علي الخلايا في القنيات المنوية، وكان التأثير متزايدا مع زيادة مدة التعرض. كما أظهرت كل أنواع الخلايا تغيرات انحلالية، وكانت الخلايا أمهات الخلايا المنوية وخلايا سرتولي أكثر الخلايا مقاومة للتأثير السمي لايزالو. وقد وجد أن الفئران النامية أكثر تأثرا من الفئران البالغة، ولكن بعد توقيف التعرض تعافت الخلايا المبطنة للقنيات المنوية في كل من الفئران البالغة والفئران النامية.

وينصح بتجنب التعرض للمستحضرات طاردة الناموس التي تحتوي علي البيريثرويدز لفترات طويلة خاصة بالنسبة للاطفال.