RESEARCH PAPER | Biological Science | Volume : 6 | Issue : 1 | JANUARY 2016 | ISSN - 2249-555X

Testicular toxicity of cypermethrin in adult and young male rats

KEYWORDS: Cypermethrin, young rats, adult rats, histopathology, testis, Spermatogenesis

Laila A. Hummdi
Biology Department- Faculty of Science for Girls- King Abdulaziz University Jeddah- Kingdom of Saudi Arabia

El-sayda H. Abdelaziz
Biology Department- Faculty of Science for Girls- King Abdulaziz University Jeddah- Kingdom of Saudi Arabia

Aisha D. Alalwani
Biology Department- Faculty of Science for Girls- King Abdulaziz University Jeddah- Kingdom of Saudi Arabia

Enaya A. Abualnasor
Biology Department- Faculty of Science for Girls- King Abdulaziz University Jeddah- Kingdom of Saudi Arabia

ABSTRACT *Pesticides are widespread synthesized substances used for public health protection and agricultural programs. However, they cause environmental pollution and health hazards. This study aimed to investigate the sub-acute effect of Cypermethrin (CYP) and to evaluate its effects of on the testis histology in adult and young male Wistar rats. To investigate the acute oral toxicity dose of CYP, 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 LD50. The acute oral LD50 value was calculated as 344.45 mg/kg. BW for adult rats and as 204.61 mg/kg. BW for young rats. Histological alterations induced in eby CYP insecticide exposure were investigated in adult and young rats by administration of low dose equal to 1/60 of LD50 and a high dose equal to 1 / 20 of LD50 for different periods 14, 28,42 days. Our results showed that many changes in testes. Testicular structure abnormalities included atrophic and distorted seminiferous tubules, deformed and disordered arrangement of germ cells, reduced germ cells, Sertoli cells and Leydig cells, vacuolization and multinucleated formations of spermatids in the CYP-treated rats. These results suggested that CYP induces impairments of the seminiferous tubules structure and spermatogenesis in the rats and therefore damages of the male reproductive system.*

INTRODUCTION

During the last decades, the undesired effect of chemical pesticides was recognized as a serious public health concern. Pyrethroid pesticides are a group of man-made products which used in agricultural, domestic, and veterinary applications (**Hashema *et al.*,2015**). However, the toxicity of pyrethroid insecticides to mammals has received much attention in recent years because animals exposed to these insecticides showed changes in their physiological activities besides other pathological features (**L´opez *et al.*,2007; Glass *et al.*, 2008**). Cypermethrin (CYP), a class II pyrethroid pesticide, first synthesized in 1974, widely used to control many pest species in agriculture, animal breeding and the household (**Elliot M *et al.*,1978; Solati *et al.*, 2010**). CYP is primarily absorbed by the gastrointestinal tract as well as by inhalation of spray mist or only simply through skin contact. Due to its lipophilic nature, CYP has been found to accumulate in body fat, skin, liver, kidneys, adrenal glands, ovaries, and brain (**Schettgen *et al* ., 2002**).

CYP was considered to be safe for mammal show ever, it was suggested that it can induce tissue damage through free radical formation and reduced antioxidant defense mechanism leading to neurotoxicity in rats (**Kamel, 2011; Sharma *et al.*, 2014**) and reproductive toxicity in mice (**Wang *et al.*, 2009**). It has been reported that long time exposure to CYP reduced serum level of progesterone in female rats (**Sangha *el al.*, 2013; Morteza *et al.*, 2014**). It was shown that the administration of CYP are associated with certain male reproductive damages including reduced sperm count, testicular lesions, sperm motility changes, sperm morphologic abnormality, infertility or sterility and genotoxic effects (**Bian *et al.*, 2004; Song *et al.*, 2008**). Therefore, the present study was designed to examine *the testes tissue toxicity of the* synthetic pesticides has been used in the Kingdom of Saudi Arabia Cypermtherin in the form of a prepared commercial product(Cypermtherin 100 EC) *on rats by identifying the* oral *half-lethal dose 50 (LD50)* and an acute, and *sub-acute doses for young and adults rats.*

MATERIALS AND METHODS

Animals

Adults Wistar male rats (*Rattus norvegicus*) was chosen at the age of 60 days with an average weight about 164.5 ± 1.225 g and young male rats weanling young at age of 22 days with an average weight about 32.5 ± 0.408 g were used in this study. Animals were supplied by the King Fahd Center for Scientific Research, King Abdel-Aziz University, Jeddah, Saudi Arabia, placed in suitable cages, maintained in constant temperature at 17°- 21°C, with proper humidity (about 60%) and illumination following the pattern of day and night. They were fed with industrial standard diet formulations that contain all essential nutrients and protein.

Chemical

The synthetic Cypermtherin Pyrethroid (α-Cyano-3phenoxy benzyl 3-(2, 2-dichloro vinyl) - 2, 2-dimethyl cyclopropane carboxylate) was purchased from the Arabian farms in Jeddah (Saudi Arabia), CAS no (23-29-495). It was used in its commercial product form Cypermtherin 100 E. C.

Acute oral toxicity

One hundred and ten (110) adult Wistar rats were divided into eleven equal groups one of them served as control group and ten experiment groups each containing ten animals and One hundred and ten (110) young Wistar rats were also divided into eleven equal as the same manner.

Volume : 6 | Issue : 1 | JANUARY 2016 | ISSN - 2249-555X

24H after acclimatization, ten graduated doses of Cypermtherin 100 EC (Cypermtherin 10%) (50, 100, 150, 200, 250, 300, 350, 400, 450/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 (**Randhawa.,2009**). 24h after administration of pesticide, animals were observed for respiratory and CNS symptoms, behavioral changes and death. LD50 was determined as bioassay experiments according to the method of **Miller and Tainter (1994)**.

Experimental design

After determination of LD50 of Cyp, 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, in order to study the impact of treatment during different periods on morphological, histological and chemical level in testes of young and adult rats. In the present study we used 180 rats from which 90 young rats and 90 adult rats. The adult rats were divided into 3 groups:

Group (G**1**), control-group, included 15 rats divided into 3 equal subgroup G**1a**, G**1b,** G**1c,** five rats each, received distilled water.

Group (G**2**), divided into 3 subgroup of five rats each, received Cyp (1/60 of LD50). Subgroups G**2a**, G**2b**, G**2c** received the same dose of CYP for 14, 28 and 42 days, respectively.

Group (G**3**), divided into 3 subgroup of five rats each, received Cyp (1/20 of LD50). Subgroups G**3a**, G**3b**, G**3c** received the same dose of CYP for 14, 28 and 42 days, respectively. This process was repeated for young rats.

Following 14, 28 and 42 days, rats were sacrificed by cervical decapitation to avoid stress. The testes samples were dissected out.

Histological study

Small pieces of testis were fixed in buffered formalin for 48 h, followed by dehydration in ascending grades of alcohol, cleared in benzene and were embedded in paraffin wax. For light microscope, sections of 3 µ thicknesses were cut and stained by hematoxylin and eosin (H & E) (**Bancroft and Gamble 2008**) .

Morphological and histological measures

Using oil emersion lens (100x) of the optical microscope sections of tissue stained by hematoxylin and eosin were examined using eye piece micrometre. We measured different diameters of seminiferous tubules and heights of germ cells layer in seminiferous tubules, as well as the diameters of the Leydig's cells 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

Data for all groups were expressed as mean ± standard deviation (X±SD). Statistical analysis methods using student t-test and Chi-square test. The results were considered to be statistically significant, highly significant, and non-significant when the P value was less than 0.05, less than 0.001,and more than 0.05, respectively.

RESULTS

Bioassay of formulated insecticide used acute toxicity

Cypermethrin did not produce any gross effect at 50mg/kg for young rats and 150 mg/kg for adult rats. For adult rats, the mortality data during determination of LD50 were 0, 1, 2, 3,5, 7, 9 and 10 against the dose were 150, 200, 250, 300, 350, 400, 450 and 500 mg/kg BW respectively. The mortality during determination of LD50 for young rats, was 0, 1, 3, 4, 6, 8, 9 against the dose were 50, 100, 150, 200, 250, 300, 350 mg/kg BW respectively and 10 against the dose was 400 mg/kg BW. The acute oral LD50 value was calculated as 344.45 mg/kg BW for adult rats and as 204.61 mg/kg BW for young rats (Tables 1,2). The current results also show sensitivity of young rats to CYP toxicity compared to adult rats, with no significant differences between them (P> 0.05) this is possibly due to the completion of liver and kidney growth among rats at the age of weaning.

Sub-acute toxicity

Based on median lethal oral dose (LD50) of pesticides used for young male and adult rats, it has been selected sub-acute low dose equal to 1/60 of median lethal oral dose and another sub-acute high equal to 1/20 of median lethal oral dose for each pesticide to administrate to young and adult rats, (Tables 3) illustrate the values of the low and high dose of CYP given daily for young, adult rats and the values of cumulative doses at the end of each experimental period (14,28,42 days). non-acute toxicity doses of pesticide for young and adult rats was estimated in the current study according to behavioral and anatomical changes, also with change in body weight and relative weight, rate of death, histological and cellular changes of testes.

Behavioral and anatomical observations on treated rats

We noted among young and adult rats treated with CYP during the first and second week of treatment unusual hyperactivity, as rats tries to cling in cage cover in order to breathe, with extension of treatment duration and increasing dose, especially in the group (G**3**) treated with high dose, we observed laxity of members, loss of ability to walk, difficulty to breathe, bled from eyes and ears for some rats, frequent urination and death of some of rats; we observed at autopsy internal bleeding between viscera and congestion of testes and liver veins and darkening of lungs color.

Body and testes weights

Results of body gain and relative testes weights of young and adult rats are shown in table 4 and table 5. The results in table 4 revealed that young rats treated with low dose of CYP (G**2**) induced significant increase in body weight gains compared to control (G**1**), while we noted a significant decrease in body weight of animals of Group (G**3**) treated with high-dose after 28, 42 days of treatment compared control group. However, tables (4,5) showed insignificant differences in relative weight of testes among young and adult rats (G**2**, G**3**) after 14 days compared to control group but the relative weight of testes were significantly decreases among the two groups treated with CYP for 28 and 42 days compared control group (G**1**). On the other hand, The table (5) shows the non-significant difference in mean body weight in the two groups of adult rats (G**3**, G**2**) after 14 days of treatment compared control group while we observed an increase in mean body weight among rats treated at the end of experimental period (42 days) compared to control (G**1**).

Histology

Control rats

The normal histological structure of rats testes which are surrounded by thick collagenous connective tissue called

Volume : 6 | Issue : 1 | JANUARY 2016 | ISSN - 2249-555X

tunica albuginea followed from the inside by vascular layer of connective tissue rich in blood vessels called tunica vasculosa. Optical microscope examination of rat testes shows several seminiferous tubules with circular and oval shape. Each seminiferous tubule is surrounded by a connective fibrous tissue, followed from the inside by long flattened cells layer with similar functionalities as smooth muscle cells called myoid cells followed from inside by basal lamina. Interstitial tissue contains numerous cells Leydig cells secrete testicular androgens, fibroblasts, leukocytes and blood vessels (Plate 1a,b). Seminiferous tubules lined by complex germinal epithelium and pyramid-shaped cells containing pale large oval nuclei called Sertoli cells (Plate 1b,c,d). Histological examination of young rats testes, aged 22 days, (G0) (Plate 1a, b) illustrates that seminiferous tubules are small and circular with an average diameter of 137.5 ± 26.01 µm and most of them does not contain lumen and are surrounded by connective tissue, also it contains few and dispersed small homogeneous cells of Leydig which contains a large nucleus and chromatin dispersed. Germ cells is made up from spermatogonia, primary spermatocytes, and a few dispersed secondary spermatocytes, spermatogonia and Sertoli cells are surrounded by basement membrane of seminiferous tubules. Two types of spermatogonia it was distinguish, the first type is characterized by a circular nucleus containing dark homogeneous chromatin and called dark type spermatogonia, the second type is characterized by a circular nucleus containing light chromatin and called light type spermatogonia. However, the (Plate 1c,d) illustrates section in adult rats control group testes at age of 75 days (G**1b**) where seminiferous tubules measuring ranging between 816±0.95µm and 412±0.30 µm; tubules contains natural germinal epithelium filled with mature spermatozoa. Increase in size ,volume and number of Leydig cells with an average diameter of 9.46 ± 1.265 µm where it gather around blood vessels and contain finely granules and empty gaps were observed. In addition to, Circular droplets around tubules between spermatozoa heads which is cytoplasm of spermatids rejected during spermatogenesis called residual bodies also been showed.

Young rats

histological examination of the Group (G**2**a) testes treated with low dose showed that testes kept their histological composition despite of germ cells reduction and separated from the spermatogonia and the basement membrane with edematous in interstitial tissue (Plate 2a).

The examination of (G**2b**) sections of treated testes showed a continuous development of testes and emergence of mature spermatocytes around seminiferous tubules and some spermatozoa inside seminiferous tubules. Separation of spermatids from spermatogonia and Sertoli cells in many tubules with acute dilation and congestion of blood vessels with infiltration of blood fluids in interstitial tissue were observed (Plate 2b). while, with extension of treatment duration a sharp expansion in some tubules ,distraction of germinal epithelium as a result of spermatocytes and spermatids deformed and necrosis leaving empty gaps were observed in (G**2c**) sections with deformed Sertoli cells nuclei and reduction in spermatogonia and mature spermatozoa, we also observed changes in Leydig cells such as in inflation of cells nuclei and decomposition of cytoplasm and lack of numbers(Plate 2c). Diversity of pathological damages in seminiferous tubules in other sections (Plate 2d) the process of spermatogenesis stopped in many tubules devoid completely of spermatozoa while containing only nuclei of Sertoli cells and multinucleated giant cells. In addition to, distort in other seminiferous tubules and lack of spermatocytes, an increase in interstitial tissue volume, acute cellular infiltration of mononuclear leukocytes with blood vessels congestion. The histological examination of testes of Group (G**3a**) treated with high dose showed decomposition of interstitial tissue around seminiferous tubules which filled with dead and sloughing spermatocytes (Plate 3a) and with extension of treatment duration in (G**3b**) observed germinal epithelium disturbance as a result of the absence of spermatids which appeared atrophied or decomposed with no appearance of mature spermatocytes compared to control group. Cytoplasmic decomposition in some spermatogonia cells and atrophy of nuclei in other spermatogonia cells with cytostatic degeneration, however Sertoli cells remain clear and intact in seminiferous tubules, also noted Leydig cells atrophy in some regions and degradation of some Leydig cells in other places (Plate 3b). Damages intensify in testes of rats treated with high dose (G**3c**) where observed retraction of seminiferous tubules and an acute decrease in the average diameters 208.889 ± 18.114 µm in length and 144.444 ± 12.664µm in width compared to control group with full stop of spermatogenesis process. (Plate 3c) showed seminiferous tubules fibrosis which appeared filled with empty gaps with lack in germinal epithelium. Sertoli cells nuclei appeared distorted and degraded and separated from the basement membrane which appeared thick when dyed with "PAS" also increased fibroblasts in edematous interstitial tissue, cellular infiltration, Leydig cells decomposed with blood vessels dilation associated with accumulation of RBCs, leading to degenerative atrophy of testes and significant decrease in relative weight compared to control group(Plate 3d) (Table 4).

Adult rats

Examination of some sections of testes of the group (G**2**a) treated low dose showed testes filled with mature seminiferous tubules containing normal germinal epithelium and spermatozoa as we observed in some sections (Plate 4a) a clear lack of spermatozoa in addition to blood vessels congestion and edematous. As noted separation of germinal epithelium from spermatogonia and Sertoli cells. Enlargement of seminiferous tubules filled with residual bodies and absence of mature spermatocytes were observed in (G**2**b) group (Plate 4b). Blood vessels congestion and interstitial edema with the emergence of eosinic drops and many gaps, lymphocytic infiltration, inflation of Leydig cells in some regions and atrophy of its nuclei in other cells has been observed in (G**2**c) group. In addition to, absence of spermatozoa in seminiferous tubules and late spermatocytes with an increase in the number of immature (circular) spermatids as well as atrophy of nuclei of spermatogonia and primary spermatocytes with decomposition (Plate 4c). Some sections of treated testes (Plate 4d) indicated acute lack of spermatozoa, decomposition of germinal epithelium surrounded by fibrous filaments with residual bodies increased, suggesting inhibition of the process of spermatogenesis as well as blood vessels congestion with Leydig cells lack. The examination of testes sections of group (G**3a**) treated with high dose shows varying degrees of necrosis in spermatids and spermatocytes. The cellular necrosis increases and gets spread in (G**3b**) where cells become spherical and separates from the germinal epithelium or decomposes (Plate 5a,b). A tightness in seminiferous tubules which contain many necrosis spermatocytes and late spermatids with absence of spermatozoa and an increase in number of Sertoli cells and lack of spermatogonia (Plate 5b) which indicates aspermatogenesis. Necrosis intensify with extension of treatment duration (G**3c**) where examination showed absence of spermatozoa and mature spermatids from the seminiferous tubules and cellular death for germinal

Volume : 6 | Issue : 1 | JANUARY 2016 | ISSN - 2249-555X

cells, spermatogonia and Leydig cells with blood vessels congestion in the interstitial tissue(Plate 5c). (Plate 5d) illustrates aspermatogenesis only in Sertoli cells, which suffers from lack in numbers and rushed away from the basement membrane of seminiferous tubules.

DISCUSSION

Cypermethrin insecticide with anti-androgen effect might present a risk to the human health and environment due to its wide use in many fields and the concern about its safety has grown (**Hu *et al.*, 2012, Hashem, *et al*,. 2015**). In the present study, young and adult rats ware used to test the toxicity of Cypermethrin on male reproductive system in adult and young rats. The acute oral LD50 value of Cyp was 204.61 mg/kg for young rats and 344.45 mg/kg for adult rats. The oral LD50 for cypermethrin in rats was reported to be 250 mg/kg (in corn oil) or 4123 mg/kg (in water) **(Meister, 1992).** EPA reports an oral LD50 of 187 to 326 mg/kg in male rats and 150 to 500 mg/kg in female rats (**U.S. EPA. 1989**). The oral LD50 varies from 367 to 2000 mg/kg in female rats, and from 82 to 779 mg/kg in mice, depending on the ratio of cis/trans- isomers present (**Who, 1989**). It was reported by **Cantalamessa (1993)** that young rats are more sensitive to toxicity of CYP compared to adult rats, and sensitivity of young rats to pesticide increase with the smaller animals. It was found that values of LD50 are equal to 14.9, 27.1, 49.3, 250 mg / kg of body weight among rats aged 8, 16, 21 days and adult treated, respectively. And this possibly may promote high sensitivity of young newborn rat to pesticides with a reduction enzymatic activity of hepatic microsomes relative to metabolic and detoxification of contaminants among young newborn rats **(Abdou *et al.*, 2012 ;Brodie, and Gillette, 2013**), or immaturation of blood testes barrier and the testes (**Setchell**, ***et al.*, 1969**) and immunity reduction (**Weir and Stewart,1993**). As many previous studies proved toxic effect of pesticides on newborn rats and young rats and mice (**Casida and al., 1983; Eriksson and Nordberg 1990**). In addition, the decrease in body weight gain in Cyp-treated young rats may be due to the combined action of neurotoxic effect and oxidative stress. Previous studies show that Cyp caused significant decrease in body weight gain in male rats (**Hussain, 2009**), female rats (**Mossa *et al.*, 2015**) and rabbits (**lakkawar, 2004**). While, increase in body weight gain in Cyp-treated adult rats is due to the deposition of pesticide in organs and body fat, where previous studies proved presence of pyrethroids pesticide residue in dairy and fatty products among animal treated Cypermtherin, Permethrin and Decamethrin (**Braun *et al.*, 1981; Croucher *et al.*, 1985**). In contrast to our study have observed dose-dependent increase testis weights of Cyp-treated rats at some acute and subacute dose levels (**Elbetieha, 2001**).

Histological structure of control rats testes is similar to normal testes of other mammals (**Berman, 2003; Junqueira and Carneiro., 2005; Walker,2010**). CYP treatment caused severe histological alterations in testis of young and adult rats. Our histological examinations showed that oral administration of CYP to young rats illustrated suppress male spermatogenesis and induce low daily sperm production. Testosterone stimulates sperm production by acting on the seminiferous tubules (**Nargund, 2015; Walker,2010**). In the current study, testicular structure abnormalities in young rats included atrophic and distorted seminiferous tubules deformed, disordered and reduced arrangement of germ cells, Sertoli cells and Leydig cells damaged, as well as vacuolization and multinucleated formations of spermatids in the CYP-treated rats. Similar results have been reported of impairments of Cypermethrin on male reproductive system (**Yousef, 2003; Wang , 2010**). It is clear also from the current study of reproductive toxicity of sub-acute repeated doses of CYP within six weeks, causes inhibition of testes function among adult rats through initiating several changes and apoptosis of germ cells, which inhibits the formation of spermatozoa and lead to testes atrophy. The current results agree with previous studies **Ahmed *et. al.*, (1989)** recorded necrosis in primary spermatocytes, separation of dead cells and sloughing healthy cells and mature spermatozoa with accumulation of residual bodies in tubules lumen among rats treated with sub-acute dose of Cypermethrin (33.5, 6.6 mg / kg of body weight) for 13 weeks. sexual maturity inhibition among young rats was attributed to chromatin decomposition of the primary spermatocytes during meiosis division as a result of pesticide combination with nuclei DNA duplication. CYP is also effective on Leydig and Sertoli cells of adult rats, **Walker,(2010)** reported that the within the seminiferous tubules, only Sertoli cells have receptors for testosterone. Thus, Sertoli cells are the major transducer of testosterone signals that are required to support germ cell survival and development. Since, the current results consonant with **(Fang, 2013)** work suggested that Cypermethrin might suppress male spermatogenesis and induce low sperm production by disturbing testosterone biosynthesis. Reduced concentration of testosterone following treatment with CYP could be considered as a cause of the degeneration of germinal epithelium which is needed for normal spermatogenesis. In the present work destruction and number reduction of Sertoli cells causes accumulation of residual bodies in the seminiferous tubules lumen of adult rats, these findings agreement with **Walker,(2010)** reported that the Sertoli cells have role in support nourish germ cells and also have phagocyte properties.

CONCLUSION

Cypermethrin is one of the pyrethroid insecticides known to be safe insecticides for non-target organisms. It has proved in current work that treatment with the sub-lethal doses of the CYP insecticide developed behavioral, body and testes weights as well as histopathlogical changes in young and adult Wister rats testes. It appears that testicular toxicity proportional with time of treatment more than amount of dosage used. Care must be taken as regards the use of CYP insecticides in agriculture, houses and veterinary purposes.

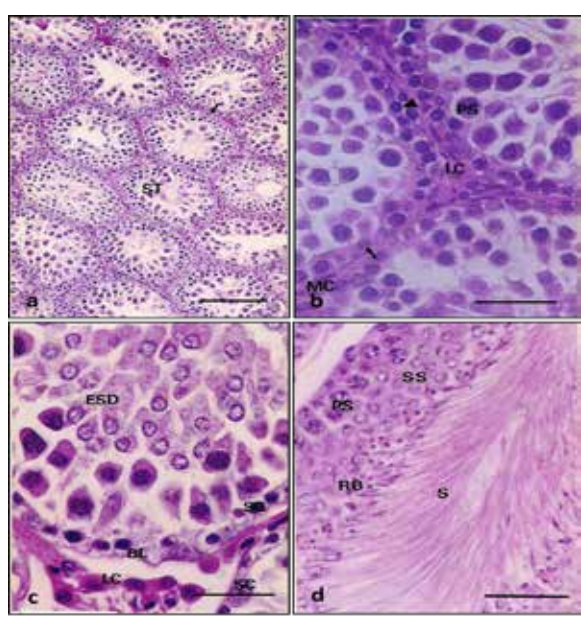

Plate (1a-d) : L.M. Testicular cross sections of control young (G_1) of male Wister rats (H & E; PAS). (a) Section of

Volume : 6 | Issue : 1 | JANUARY 2016 | ISSN - 2249-555X

control testis (G_{1_0}) at 22 day age. Note small seminiferous tubules (ST) surrounded by fibrous connective tissue layer (arrow); scale bar = 100 µm. (b) High power from (1a) showing pale spermatogonium (arrow); dark spermatogonium (head arrow) & primary spermatocytes (PS). Note myoid cell (MC) & narrow intertubular space contain interstitial (Leydg's) cells (LC); scale bar = 50 µm. (c) Section of control testis (G_{1a}) at 36 days age. showing 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. (d) Section of control adult (G_{1C}) at 75 days age showing spacious lumen of seminiferous tubules filled with spermatozoa (S) & normal stratified germinal epithelium. Note primary spermatocytes (PS); secondary spermatocytes (SS) in cellular association and many PAS positive droplets near the luminal edge which represent residual bodies (RB); PAS; scale bar = 20µm.

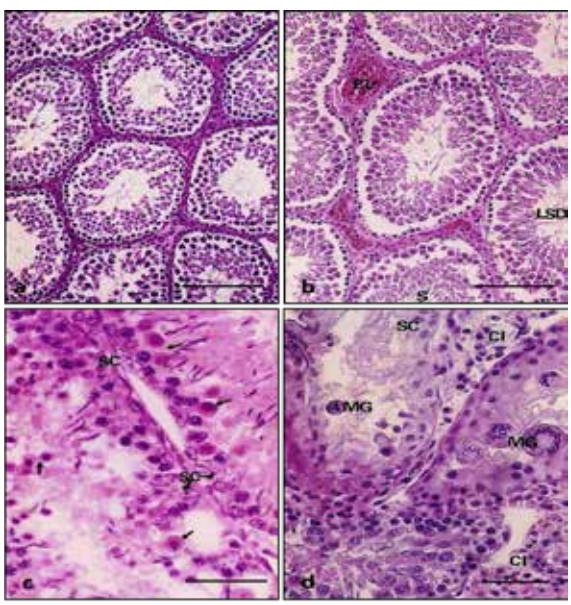

Plate (2a-d) : L.M. Testicular cross sections from young rat group (G_2) treated with low dose of cypermethrin (H. & E.).(a) : Section of treated testis (G_{2a}) showing normal testicular architecture with slightly disturbed germ cell arrangment; scale bar = 100 µm. (b) : Section of treated testis (G_{2b}) showing dilatation & congestion in blood vessels (BV); separation of germ cells from spermatogonia and sertoli cells in seminiferous tubules; Note late spermatids (LSD) & spermatozoa (S); scale bar = 100 µm. (c) : Section of treated testis (G_{2c}) showing dilated and vacuolated tubules contain necrotic germ cells (arrows) with darkly stained nucleus and acidophilic cytoplasm. Note, deformed elongated late spermatid, spermatozoa and sertoli cell nuclei (SC); scale bar = 20 µm. (d) : Section of treated testis (G_{2c}) showing tubules with no evidence of spermatogenesis but contain only sertoli cells (S) and multinucleated giant cells (MG); other tubules showed hypospermatogenesis. Note, cellular infilteration (CI) ; scale bar = 100 µm.

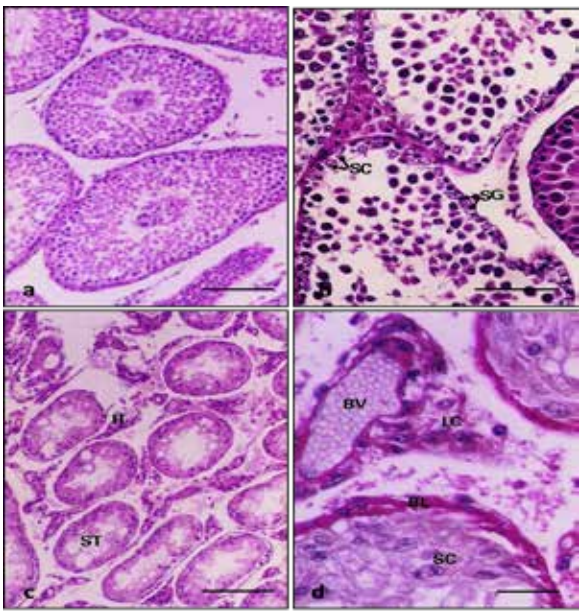

Plate (3a-d) : L.M. Testicular cross sections of young rat group (G_3) treated with high dose of cypermethrin (H. & E. & PAS).(a) : Section of treated testis (G_{3a}) showing slighty disturbed germinal epithelium and desquamation of the normal intact & necrotic germ cells into the tubular lumen; scale bar = 100 µm. (b) : Section of treated testis (G_{3b}) showing a dramatic loss of normal germ cell associations of germinal epithelium due to damage and necrosis. Note, cytostatic damage of spermatogonia (Sg) but sertoli cells (Sc) remain intact; scale bar = 50 µm. (c) : Section of treated testis (G_{3c}) showing massive atrophy of seminiferous tubules (ST) associated with missing of all germ cells. Note increased amounts of interstitial tissue (IT); scale bar = 100 µm. (d) : Enlarged part from previous section showing atrophoid seminiferous tubules with thickened basal lamina (BL) and deformed sertoli cell (SC) nuclei which pused out from basal lamina. Note dilated and congested blood vessel (BV) and degenerated leydg's cells (LC) ; PAS scale bar = 20 µm.

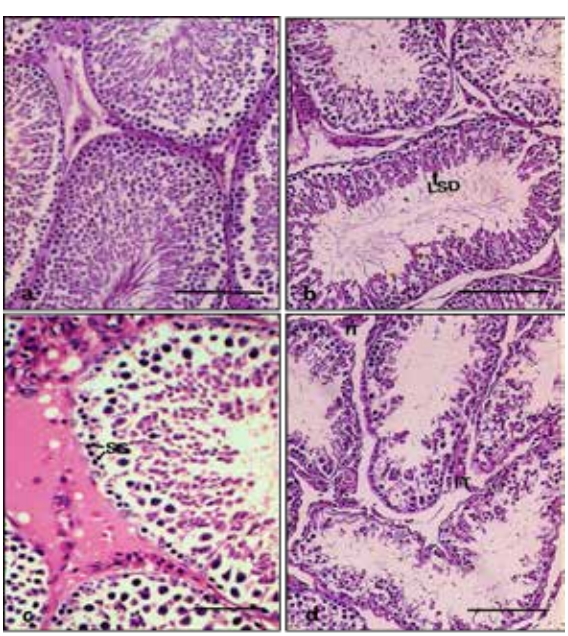

Plate (4a-d) : L.M. Testicular cross sections from adult

Volume : 6 | Issue : 1 | JANUARY 2016 | ISSN - 2249-555X

rat group (G_3) treated with high dose of cypermethrin (H. & E.). (a) : Section of treated testis (G_{2a}). Note tubules populated with all stages of spermatogenesis but cellularity is relatively low (hypospermatogenesis). Note also oedemotous interstitium associated with inflammatory cell infilteration; scale bar = 100 µm. (b) : Section of treated testis (G_{2b}). Note tubules with spacious lumen free from spermatozoa & surrounded by late spermatids (LSd). Note disorganized germinal epithelium; scale bar = 100 µm. (c) : Section of treated testes (G_{2c}) showing loss of spermatozoa and late spermatids from tubular lumen; a loose and disorganized epithelium. Note; oedematous interstitium associated with cellular infelteration & eosinophilic droplets; Note also necrotic spermatogenia (Sg) and damaged sertoli cells; scale bar = 50 µm.(d) : Section of treated testis (G_{2c}) showing dilated and irregular surfaces tubules. Note, massive loss of germ cells and spermatozoa. Note congested capillaries; and damaged interstitial tissue (IT); scale bar = 100 µm.

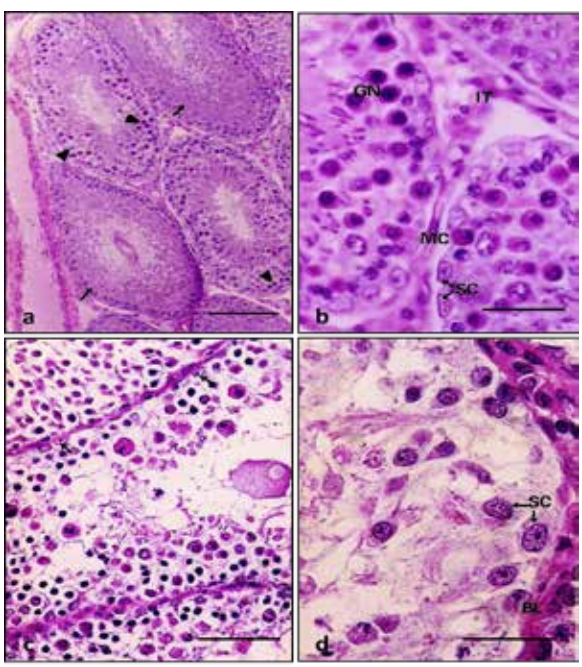

Plate (5a-d) : L.M. Testicular cross sections from adult rats (G_3) treated with high dose of cypermethrin (H. & E.). (a) : Section of treated testis (G_{3a}) showing normal stratified germinal epithelium in some tubules (arrow) and heavy cellular necrosis in other (head arrow) ; scale bar = 100µm. (b) : High power from (a) showing germ cell necrosis (GN) with darkly stained nuclei and acidophilic cytoplasm; proliferated sertoli cells (Sc). Note also separation of myoid cell layer (MC) from basal lamina of ST and damaged interstitial tissue cells (IT) ; scale bar = 20 µm. (c) : Section of treated testis (G_{3c}) illustrating massive germ cell and spermatogonial necrosis and degeneration, but still sertoli cell nuclei easily demarkated; (arrow) scale bar = 50 µm. (d) : Section of treated testis (G_{3c}). Note part of atrophoid seminiferous tubules showing loss of lining germinal epithelium. Note also intact sertoli cells pushed out from thickened basal lamina (Bl) and cellular debris scattered in lumen; scale bar = 20 µm.

Table (1) The percentage mortality recorded 24 hr after insecticides treatment in young and adult male rats. Cypermethrin 100 EC (10% cypermethrin)

Dose		Youngs			Adults	
mg/kg/	N. of	Mortality		N. of	Mortality	
BW	individuals	N	%	individuals	N	%
50	10	0	-	10	0	-
100	10	1	10	10	0	-
150	10	3	30	10	0	-
200	10	4	40	10	1	10
250	10	6	60	10	2	20
300	10	8	80	10	3	30
350	10	9	90	10	5	50
400	10	10	100	10	7	70
450	10	10	100	10	9	90
500	10	10	100	10	10	100
Control	10	10	-	10	0	-

Table (2): Acute toxicity of cypermethrin in rats.

Treated	Cypermethrin
animals	24 hr LD50 (mg/kg BW)
Youngs	204.61 (193.16 – 216.74)*
Adults	344.45 (326.99 – 362.84)*
Sign. test	χ^2 = 3.3374; P = 0.132774

* Values in parenthesis are the 95% confidence limits
χ^2 = Chi square test; P>0.05 not significant

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

Young	No. of	Dose (mg/kg	Subgroups		Period	Cumulative	Mortality	
Groups	animals	BW per day)	Abbrev.	N	of exp. (days)	dose (Mg\kg B.W.)	N	%
G_1	15	-						
Control		-						
			G_1a	5	14	-	-	
			G_1b	5	28	-	-	
			G_1c	5	42	-	-	
G_2	15							
Low dose		3.4102 mg/kg B.W./day	G_2a	5	14	47.7428	-	
			G_2b	5	28	95.48561	1	20
			G_2c	5	42	143.22821	2	40

Volume : 6 | Issue : 1 | JANUARY 2016 | ISSN - 2249-555X

G_3 High dose	15	10.2306 mg/kg B.W./day	G_3a	5	14	143.228	1	20
			G_3b	5	28	286.4568	2	40
			G_3c	5	42	429.6852	2	40
Adult	**No. of**	**Dose (mg/kg**	**Subgroups**		**Period**	**Cumulative**	**Mortality**	
Groups	**animals**	**BW per day)**	**Abbrev.**	**N**	**of exp. (days)**	**dose (Mg\kg B.W.)**	**N**	**%**
G_1	15	-						
Control		-						
			G_1a	5	14	-	-	
			G_1b	5	28	-	-	
			G_1c	5	42	-	-	
G_2	15							
Low dose		5.7487 mg/kg B.W./day	G_2a	5	14	80.4818	-	
			G_2b	5	28	160.9639	-	
			G_2c	5	42	241.445	1	20
G_3	15							
High dose		17.222 mg/kg B.W./day	G_2a	5	14	241.445	1	20
			G_2b	5	28	482.216	1	20
			G_2c	5	42	723.324	2	40

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

Body and organ weights / Sub-groups and treatment days Control G_1		Body weight (g)			Relative testes weight (g)		
		treated			treated		
		G_2 Low dose	G_3 High dose	Control G_1	G_2 Low dose	G_3 High dose	
	Avg.	32.500	32.500	32.500	1.0985		
	±SD	0.408	0.707	0.707	0.012		
0	T		0	0			
	P		1	1			
	Avg.	119.5	120.667	114.667	1.797937	1.948003	1.792108
(a)	±SD	2.0412	2.62467	3.68179	0.071793	0.129624	0.147129
14	T		-1.7722	1.62369		-3.64288	0.038955
	P		0.15150	0.17976		0.66951	0.486232
	Avg.	156.5	157.33	149.833	1.790064	1.2704	1.223613
(b)	±SD	1.2247	0.89898	1.31233	1.118359	0.11732	0.056069
28	T		-2.2206	5.25226		18.85002	12.77644
	P		0.08226	0.00629**		0.001401**	0.003035**
	Avg.	206.500	209.500	194.14	1.8077	1.5059	1.3768
(c)	±SD	0.408	0.4084	1.4626	0.009991	0.0524	0.0241
42	T		2.77645	7.3485		4.238	9.4608
	P		0.1830	0.008**		0.0133*	0.0007**

Significant levels : - $p > 0.05$ not significant - $P < 0.05$ * significant; $p < 0.01$ ** or $P < 0.001$ ** highly significant. T-student t-test; ± SD : standard deviation

Volume : 6 | Issue : 1 | JANUARY 2016 | ISSN - 2249-555X

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

Body and organ weights / Sub-groups and treatment days ControlG1		Body weight (g)			Relative testes weight (g)		
		treated			treated		
		G_2 Low dose	G_3 High dose	Control G_1	G_2 Low dose	G_3 High dose	
	Avg.	194.500	194.333	194.833	1.993		
	± SD	1.225	1.027	1.650	0.042		
0	T		0.14744	-0.2290			
	P		0.88992	0.8298			
	Avg.	222.333	221.500	286.66	1.733454	1.726219	1.708934
(a)	± SD	6.128	7.757	4.643	0.066049	0.097419	0.071128
14	T		0.11922	3.12701		0.063867	0.261265
	P		0.91085	0.7353		0.477443	0.409166
	Avg.	238.83	242.5	238.83	1.653831	1.473536	1.274729
(b)	± SD	1.0274	4.89898	1.0274	0.010058	0.024722	0.110876
28	T		-3.8612	-0.4376		8.112236	5.299639
	P		0.01813*	0.68427		0.007429**	*0.016905
	Avg.	253.17	266.833	259.5	1.659083	1.2524	1.1669
(c)	± SD	1.9293	3.11805	2.2640	0.012698	0.032210	0.01142
42	T		-5.2712	3.8616		5.05788	9.5899
	P		0.00621**	0.01814*		0.01847	0.00535**

Significant levels : - $p > 0.05$ not significant. - $P < 0.05$ * significant; $p < 0.01$ ** or $P < 0.001$ ** highly significant T-student t-test; ± SD : standard deviation

REFERENCE

Abdou. H. M, Hussien. H. M, and Yousef. M. I. 2012. Deleterious effects of cypermethrin on rat liver and kidney: protective role of sesame oil. Journal of Environmental Science and Health. Part B: Pesticides, Food Contaminants, and Agricultural Wastes, vol. 47, no. 4, pp. 306–314. Ahmed, N., Gupta, P. K., and K. C. Cieorge. 1989 . Subacute toxicity of cypermethrin in rats. J. Environ. Biol. 10 (3) : 309 – 317 . Bancroft, J.D., Gamble, M., 2008. Theory and Practice of Histological Techniques, 6th ed. Churchill Livingstone, Scotland, London, pp. 125–138. Berman, I., 2003. Color atlas of basic histology. 3rd edition. Lange Medical Books/ McGraw- Hill Inc. USA. Pp 272. Bian, Q., Xu, L.C., Wang, S.L., et al., 2004. Study on the relation between occupational fenvalerate exposure and spermatozoa DNA damage of pesticide factory workers. Occupational and Environmental Medicine Med. 61, 999–1005. Braun, H. E; Surgeoner, G. A; Stanek, J. and W. E. Ralley .1981. Efficacy and dissipation of permethrin for the control of the northern fowl mite in hens. Canad . Net. J. (22) : 291 – 294 . Brodie, B.B and Gillette, J.R., 2013. Drugs and Enzymes. 1st Proceedings of The Second International Pharmacological Meeting, Vol. 4,Pp 512. Cantalamessa, F. 1993. Acute toxicity of two pyrethroids, permethrin, and cypermethrin in neonatal and adult rats. Archives of Toxicology. 67(7):510-3. Casida, J.F . 1983. Development of synthetic insecticides from natural products: case history of pyrethroids from pyrethrin in natural products for in novative pest management .EDS.D.L. Whitehead and W.S .Bowers .New York. Pergram on press . PP 127 –151 . Croucher , A; Hutson , D. H; and G .Stoydin .1985. Excretion and residlues of the pyrethroid insecticide cypermethrin in lactating cows .Pest . Sci . (16) : 287 – 301 . Elbetieha. A, Da'as. S. I, Khamas. W, and Darmani. H. 2001. Evaluation of the toxic potentials of cypermethrin pesticide on some reproductive and fertility parameters in the male rats. Archives of Environmental Contamination and Toxicology, vol. 41, no. 4, pp. 522–528. Elliott, M., Janes, N.F., Pulman, D.A., Gaughan, L.C., Unai, T., Casida, J.E. 1976. Radiosynthesis and metabolism in rats of the 1R isomers of the insecticide permethrin. Agricultural and Food Chemistry. 24, 270–276. Ericsson,P. and A.Nordberg .1990. Effects of two Pyrethroids, bioallethrin and deltamethrin on subpopulations of muscarinic and nicotinic receptors in the neonatal mouse brain. Toxicol. Appl. Pharmacol. (102) : 456 – 463 . Fang, L.Y., Chen, P., Xia, H.U.J., Jing, L.I., Chun, X.L. 2013. Effects of cypermethrin on male reproductive system in adult rats. Biomed. Environ. Sci. 26, 201–208. Glass. R., 2008. Chronic and long-term effects of pesticides use in agriculture: current knowledge and limits. Toxicology Letters. vol. 180, p. S21. Hashema, H. E., Abd El-Haleema, M. R., Abassb, M. A.. 2015. Epithelial and stromal alterations in prostate after cypermethrin administration in adult albino rats (histological and biochemical study). Tissue and Cell. Article in press. Hu, J.X., Li, Y.F., Pan, C., Zhang, J.P., Wang, H.M., Li, J., Xu, L.C. 2012. Anti-androgen effects of cypermethrin on the amino- and carboxyl-terminal interaction of theandrogen receptor. Toxicology 292, 99–104. Hussain. S. Khan. M. Z, Khan. A, Javed. I, and Asi. M. R. 2009. Toxico-pathological effects in rats induced by concurrent exposure to aflatoxin and cypermethrin. Toxicon, vol. 53, no. 1, pp. 33–41. Junqueira, L.C., Carneiro. J. 2005. Basic histology: text and atlas. 11th.McGraw-Hill.Pp435. Kamel, A.M.F. 2011. Histologic study on the protective effect of a-lipoic acid in sciaticnerve neurotoxicity induced by cypermethrin in albino rats. Egypt. Journal of Histology. 34,218–230. Hummadi, A. L. 2012. Adverse Effects of Soriatane on Rats Enterocytes: Light Microscopy and Ultrastructural Studies. J Cytol Histol .,3:1, 133-138. Lakkawar A. W, Chattopadhyay. S. K, and Somuanshi. R. 2004. Experimental cypermethrin toxicity in rabbits—a clinical and patho-anatomical study. Folia Veterinaria, vol. 48, pp. 3–8. L´opez. O, Hern´andez. A. F, Rodrigo.L et al., 2007. Changes in antioxidant enzymes in humans with long-term exposure to pesticides. Toxicology Letters, vol. 171, no. 3, pp. 146–153. Meister, R.T. 1992. Farm Chemicals Handbook '92. Meister Publishing Company,Willoughby, OH. Miller LC, Tainter ML. Graphical method for determination of LD50. 1944. Proceedings of the Society for Experimental Biology and Medicine, 57, 261. Morteza Molavi, Mazdak Razi, Hassan Malekinejad, Amir Amniattalab, Hamed Rezaie. 2014. Vitamin E improved cypermethrin-induced damages in the ovary of rats; evidence for angiogenesis and p53 involvement. Pesticide Biochemistry and Physiology 110, 27–35. Mossa, A.H., Ibrahim, F.M., Mohafrash, S.M., Aboubaker, D.H. and El Gengaihi., S. 2015. Protective effect of ethanolic extract of grape pomace against the adverse effects of cypermethrin on weanling female rats. Evidence-based complementary and alternative medicine. Article ID 381919, 10 pages. Nargund,V.H., 2015. Effects of psychological stress on male fertility. Nature Reviews Urology. 12: 373–382 Randhawa MA. 2009. Calculation of LD50 values from the method of Miller and Tainter, 1944. The Journal of Ayub Medical College Abbottabad; 21:184–5. Sangha. G.K., Kaur. K., Khera. K.S. 2013. Cypermathrin-induced pathological and biological changes in reproductive organs of female. Environmental Biology. 34 99–105. Schettgen T, Heudorf U, Drexler H et al. 2002. Pyrethroid exposure of the general population: Is this due to diet? Toxicology Letters 134: 141–145. Setchell , B .P ; Scott , T. w ; Voglmayer , J . K ; and G . M . H. 1969. Characteristics of testicular spermatozoa and the fluid which transports them into the epididymis . Biol . Reprod . (1) : 40 – 66 . Sharma, P., Firdous, S., Singh, R., 2014. Neurotoxic effect of cypermethrin and protective role of resveratrol in Wistar rats. International Journal of Nutrition, Pharmacology, Neurological Diseases. 4,104–111. Solati, J., Hajikhani, R., Zaeim, R.T., 2010. Effects of cypermethrin on sexual behaviourand plasma concentrations of pituitary–gonadal hormones. International journal of fertility & sterility.4, 23–28. Song, L., Wang, Y.B., Sun, H., et al., 2008. Effects of fenvalerate and cypermethrin on rat sperm motility patterns in vitro as measured by computer-assisted spermanalysis. Journal of Toxicology and Environmental Health. 71, 325–332. U.S. Environmental Protection Agency. 1989. Pesticide Fact Sheet Number 199: Cypermethrin. Office of Pesticides and Toxic Substances, Washington, DC, 2-9 Walker,W.H., 2010. Non-classical actions of testosterone and spermatogenesis. Philosophical Transactions of the Royal Society, B., 365: 1557–1569. Wang H, Wang Q, Zhao XF, et al., 2010. Cypermethrin exposure during puberty disrupts testosterone synthesis via down regulating StAR in mouse testes. Archives of Toxicology; 84, 53-61. Wang, X.Z., Liu, S.S., Sun, Y., Wu, J.Y., Zhou, Y.L., Zhang, J.H., 2009. Beta-cypermethrinimpairs reproductive function in male mice by inducing oxidative stress. Theri-ogenology 72, 599–611. Weir , D. M ., J. Stewart .1993. Immunology . 7th ed . Churchell living stone . printed in Singapore. Pp. 372. WHO. 1989. Organization Environmental health criteria 82 : cypermethrin . Geneva , World health organization , pp 154 . Yousef, M.I., El-Demerdash, F.M., Al-Salhen, K.S. 2003. Protective role of isoflavones against the toxic effect of cypermethrin on semen quality and testosterone levels of rabbits. Journal of Environmental Science and Health, B; 38, 463-78.