

HEMATOLOGICAL AND HISTOPATHOLOGICAL CHANGES IN THE TESTES AND SEMINAL VESICLE OF RATS FOLLOWING REPEATED EXPOSURE TO LAMBDA-CYHALOTHRIN

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Abstract

Lambda-cyhalothrin (LCT) is a type II pyrethroid insecticide widely used in pest management. The purpose of the current study was to evaluate the toxic effects of LCT on hematological and histopathological changes in the testis and seminal vesicles of rats after subacute exposure.

Twenty-eight 6-week-old male albino rats *Rattus norvegicus* were randomly assigned to four groups. Group 1 was the control group, which received distilled water. The experimental groups 2, 3 and 4 received by gavage 20.4, 30.6 and 61.2 mg/kg body weight, respectively, of LCT over 28 days. The effects of the insecticide on various hematological parameters were evaluated at 14 and 28 days. In the plasma, residual concentrations of lambda cyhalothrin mixture, LCT 1R, cis, S cyhalothrin (1) and 1S, cis, R, cyhalothrin (2) were determined by GC/MS/MS. Histopathological studies were carried out in the testes and seminal vesicles at the end of the experiment.

The body weight, feed intake, the absolute and relative weights of testes and seminal vesicles were significantly decreased. Lambda-cyhalothrin, as a pyrethroid insecticide, induced significant decrease ($p < 0.005$) in blood RBCs, Hb and MCV count after 14 and 28 days. In the rat plasma samples after 28 days, residual concentrations of LCT 1R, cis, S cyhalothrin (1) and 1S, cis, R, cyhalothrin (2) were clearly detected.

All experimental animals showed dose-dependent organ pathologies. Our histological results showed congestion and damaged seminiferous tubule which showed complete spermatogenic arrest, less lumen formation contained cellular debris and less interstitial cells or Sertoli cells in the testis of the rat treated with LCT. Atypical hyperplasia and degeneration of the seminal vesicles was found in the high dose (61.2 mg/kg) LCT treated rats when compared with that of controls. It can be concluded that lambda cyhalothrin is highly toxic for rat testis and seminal vesicle.

Key words: lambda-cyhalothrin, biochemical, histopathology, testis, seminal vesicles

1. INTRODUCTION

Although pesticides have largely benefited the human life through enhancement of agricultural products and controlling infectious diseases, their extensive use, in turn, has offended human health from side to side of occupational or environmental exposures.

Long-term contact to pesticides can harm human life and can disturb the function of different organs in the body, including nervous, endocrine, immune, reproductive, renal, cardiovascular, and respiratory systems (Mostafalou and Abdollahi, 2013).

Pyrethroids are synthetic analogues of pyrethrins I, the naturally occurring insecticides extracted from the dried flowers of *Chrysanthemum cinerariaefolium* (Soderlund et al., 2002). They are often used in household sprays, aerosol bombs, insect repellents, pet shampoos, and lice treatments (Casida, 1980; Righi & Palermo, 2005). Pyrethroids are categorized into type I and type II, which differ in chemical structure and mechanisms of action (Breckenridge et al, 2009).

Lambda-cyhalothrin ($C_{22}H_{19}ClF_3NO_3$) (LCT), is a halogenated pyrethroid comprising a 1:1 mixture of two stereoisomers, namely, [(S)-a-cyano-3-phenoxybenzyl-(Z)-(1R,3R)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl cyclo propane carboxylate and (R)-a-cyano-3 phenoxybenzyl- (Z)-(1S,3S)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate] (Colombo et al., 2013).

The toxicity of pyrethroid insecticides to mammalian animals has received much attention in recent years because animals exposed to these insecticides exhibited changes in their physiological activities beside other pathological features. Therefore, using products containing these compounds will expose people to these chemicals (Dahamna et al., 2009).

Conventional pesticide residue analyses of pyrethroids mostly focus on chromatographic separation using high performance liquid chromatography, gas chromatography (GC), or coupled column liquid chromatography/mass spectrophotometry (Cheng et al., 2009 ; Turrillas et al., 2005).

The current study was performed to assess the adverse effect of lambda cyhalothrin (LCT) on reproductive organs, testes and seminal vesicle, of male fertility during subacute LCT exposure and to further investigate the dose effect of LTC on hematological parameters and plasma residue levels of the two isomers of LCT (1R, cis, S and 1S, cis, R).

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

A pure reference standard of lambda cyhalothrin (>98.9%, purity, Syngenta Corp., Greensboro, NC, USA) was obtained from the Ministry of Agriculture and Rural Development (*Direction de la Protection des Végétaux et des Contrôles Techniques*, DPVCT) of Algeria. A commercial formulation of LCT named Karate ZTM (λ -cyhalothrin 50 g/L; Syngenta Corp.) was used in the experiments. All other chemicals were of reagent grades and obtained from commercial sources: Biosolve Chimie (Valkenswaard, Netherlands) and BIOLABO SA (Maizy, France).

2.2 Animals

Twenty-eight Male albino rats weighing 160 ± 10 g obtained from CRD-SAIDAL (El Harrach, Algeria) were used in this study. The animals were housed in plastic cages and accustomed to laboratory conditions for a week before starting the experiment. Rats were fed on standard food pellets (ONAB: *Office National des Aliments de Bétail*, Algiers) and tap water *ad libitum*. The rats were housed at $22 \pm 3^\circ\text{C}$ and in daily dark/light cycle. The experimental work on rats was performed according to the standard procedures laid down by OECD guideline 407 for repeated dose 28-day oral toxicity studies in rodents (OCDE, 2003).

2.3 Experimental design

Animals were divided into four groups, seven animals each as the following:

Group 1: Rats were served as control and given distilled water. Groups 2, 3, and 4 received an aqueous solution of LCT at three different doses: 20.4 mg/kg b.w. (Low dose), 30.6 mg/kg b.w. (Middle dose), and 61.2 mg/kg b.w. (High dose), respectively, daily for 28 days by gavage. Animals were weighed daily and the dose was adjusted accordingly. At the end of the experiments, blood samples were collected for hematological and analytical evaluations. Rats were euthanized by cervical decapitation under light diethyl ether anesthesia.

The testes and seminal vesicles were carefully dissected out and weighed in grams (absolute organ weight). The relative testes weight of each animal was then calculated as follows:

Relative organ weight = $\frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$

Body weight of rat on sacrifice day (g)

2.4 Haematology

This study involved two exposure periods: a 14-day and a 28-day study. After 14 days of administration of LCT, blood samples were obtained from the retro-orbital sinus for haematological evaluation.

Blood samples for haematological analyses were collected in EDTA coated tubes, and assayed using a Haematology Analyzer (Sysmex automatic counter) for the following parameters: haematocrit (Hct), haemoglobin concentration (HGB), red blood cell count (RBC), platelet count (PLT), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC).

2.5 Plasma lambda-cyhalothrin concentration

At the end of exposure period, blood samples were withdrawn from the animals under ether anesthesia by puncturing the retro-orbital venous plexus of the animals with a fine sterilized glass capillary. Blood was collected into heparinized tubes and centrifuged at 3000 rpm for 20 minutes to separate the plasma. The plasma was kept in a deep freezer (-20°C) until analyzed within one week.

The analysis of rat plasma for lambda cyhalothrin was performed in a GC-MS/MS system, coupled with a tandem mass spectrometer (Quattro Micro TM GC MICROMASS®; Waters Corp., Milford, MA, USA). Chromatographic separation was achieved with a fused silica capillary column AT5-ms (ref: 15807; Alltech, Templemars, France). One µL was injected in splitless mode at an injection temperature of 250°C. The initial oven temperature of 25°C was kept for 2 min, increased to 250°C, and then kept at this temperature for 8 min. Retention times were 8 min for the standard used for LCT quantification (PCB -166), and 12.40 min and 12.85 min for cyhalothrin 1 and cyhalothrin 2 respectively. All data collected were acquired using Mass Lynx TM NT 4.1 software.

2.6 Histopathological studies

Testes and seminal vesicles of each rat were removed and fixed in 10% buffered formalin. The fixed tissues were processed through the conventional paraffin embedding technique. Thin sections of 2-3 µm were cut, and stained with hematoxylin and eosin for pathological evaluation under the microscope.

2.7 Statistical analysis

The results were expressed as mean ± S.D. All data were done with Statistica 10. The results were analyzed using one way analysis of variance (ANOVA) followed by Duncan's test for comparison between control and different treatment groups. Statistical significance was set at $p \leq 0.05$.

3. RESULTS

3.1 Evaluation of body and organ weights

A significant difference in the absolute and percentage of body weight gain at the end of the experimental was observed in treated LCT 30.6 and 61.2 mg/kg body weight compared to the other groups (Table 1). A decrease in body weight and average feed intake were observed for rats treated with a high dose of LCT compared with control rats.

Similarly, a significant decrease in the absolute and relative weights of seminal vesicle and right and left testes was observed (Table 2).

Table 1: initial, final and percentage body weight gain and average feed intake in rats from control and LCT-treated groups (20.4, 30.6 and 61.2 mg/kg b.w.).

Groups	Body weight (g)			
	Initial	Final	% BWG	Average feed intake
Control ^c	167±10	232±13	28	20.45±0.38
Low LCT ^{a, c}	163±8	230±16	29	19.21±0.65
Mid LCT ^{b, c}	162±8	220±16*	26*	16.51±25*
High LCT ^{d, c}	165±10	198±9*	16*	13.11±32*

a **20.4** mg of LCT/kg/day.

b **30.6** mg of LCT/kg/day.

d **61.2** mg of LCT/kg/day.

c Results are given as a mean±SD ($n = 7$).

* Statistically different from the control group ($P < 0.05$).

Table 2: Absolute and relative testes and seminal vesicle weights (g) in rats from control and LCT-treated groups (20.4, 30.6 and 61.2 mg/kg b.w.).

Seminal vesicle and Testes Weights (g)				
Dose	Organs Weight	Seminal vesicle	Testes (Right)	Testes (Left)
Control ^c	Absolute	1,15 ±0,27	1,24± 0,27	1,23 ± 0,16
	Relative	0,49 ±0,12	0,53± 0,12	0,53 ± 0,06
Low LCT ^{a, c}	Absolute	1,14± 0,23	1,13± 0,25*	1,16± 0,21*
	Relative	0,50± 0,10	0,50± 0,11*	0,51± 0,12*
Mid LCT ^{b, c}	Absolute	1,18± 0,15*	1,17± 0,14*	1,16± 0,14*
	Relative	0,52± 0,10	0,51± 0,06*	0,51± 0,06*
High LCT ^{d, c}	Absolute	1,21 ±0,14*	0,88 ±0,09*	0,89 ±0,08*
	Relative	0,61 ±0,07*	0,44 ±0,05*	0,44 ±0,06*

^a **20.4** mg of LCT/kg/day.

^b **30.6** mg of LCT/kg/day.

^d **61.2** mg of LCT/kg/day.

^c Results are given as a mean±SD (*n* = 7).

* Statistically different from the control group (*P* < 0.05).

3.2 Haematological results

The effects of LCT on haematological parameters in the rats are given in Table 3. In LCT-treated rats, (RBC) red blood cell, (Hct) hematocrit, (MCV) mean corpuscular volume, and (MCH) mean corpuscular hemoglobin and (PLT) platelet were significantly different when compared with those of controls in a dose- and time dependent manner. However, treatment with LCT did not affect blood (HGB) hemoglobin and (MCHC) mean corpuscular hemoglobin concentration of LCT-treated rats compared to control (Table 3).

Table 3 Effects of lambda cyhalothrin on blood haematological parameters of control and LCT-treated rats.

Blood parameters	Period	Control ^c	Low LCT ^{a, c}	Mid LCT ^{b, c}	High LCT ^{d, c}
RBC(10 ⁶ /μL)	14	8,01±0,52	8,19±0,67	8,23±0,55	8,03±0,54
	28	8,18±0,25	8,26±0,31	8,10±0,87	7,05±0,63*
Hct (%)	14	46,04±1,90	48,48±3,17*	47,26±3,73*	45,42±1,39*
	28	48,22±1,90	49,04±3,30*	48,08±5,12*	46,20±3,11*
HGB (g/dL)	14	14,52±0,43	15,14±0,75	14,52±1,70	14,42±0,52
	28	14,48±0,78	14,94±1,36	14,76±1,36	14,20±0,80
MCV (fL)	14	59,84±2,03	57,86±2,23*	57,52±4,97*	56,68±3,13*
	28	58,96±2,48	57,28±3,37*	59,42±3,64*	61,25±0,98*
MCH (pg)	14	18,90±1,04	18,06±0,94*	17,68±2,31*	18,02±1,75
	28	17,70±1,05	17,44±1,48	18,28±1,13*	18,85±0,51*
PLT(10 ³ /μL)	14	910,40±99,32	777,80±99,39*	806,4±98,7*	915,4±91,2
	28	958,60±201,54	886,00±96,93*	858,20±142,5*	910,00±3,4*
MCHC(g/dL)	14	31,56±0,74	31,26±0,65	30,66±1,77	31,78±1,32
	28	30,02±0,58	30,42±0,88	30,78±0,90	30,75±0,28

Abbreviations used: Hct = hematocrit, HGB = hemoglobin concentration, RBC = red blood cell, PLT = platelet, MCH = mean corpuscular hemoglobin, MCV= mean corpuscular volume, and MCHC = mean corpuscular hemoglobin concentration.

3.3 Plasma lambda-cyhalothrin concentration

Lambda cyhalothrin was found in the plasma samples analyzed but in very low concentrations below the quantification limit (1 ng/g) after 14 days of treatment. However, on day 28 residual concentrations of the two isomers of LCT (1R, cis, S and 1S, cis, R) were found in all plasma of rats treated with 30.6 mg/kg [7.21±2.45 and 4.96±4.09 ng/mL] and 61.2 mg/kg [24.87±4.51 and

31.08±2.43 ng/mL] of LCT. Two peaks were observed in the chromatograms for lambda cyhalothrin due to the separation of its diastereoisomers Figure 1.

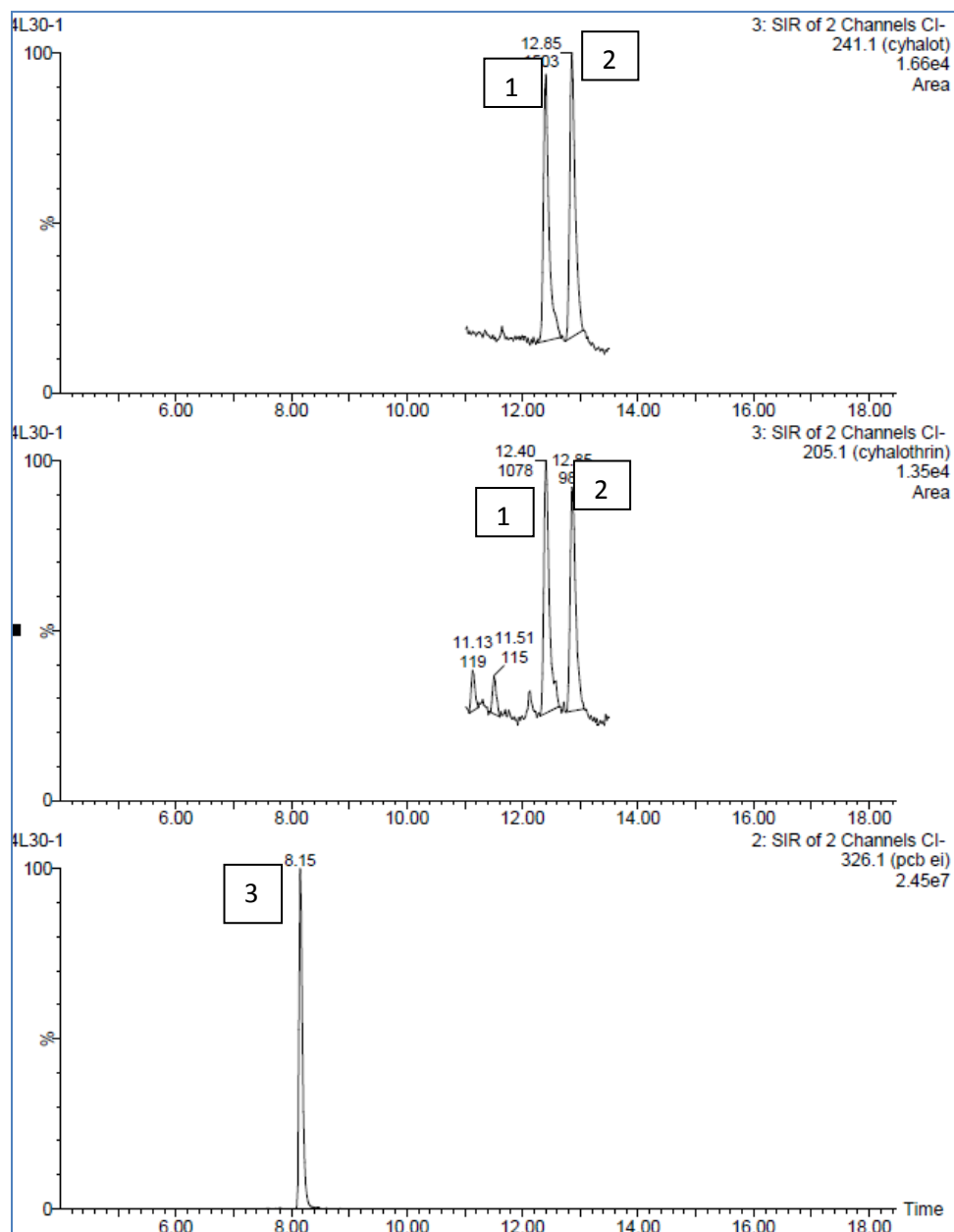


Figure 1. GC-MS/MS, chromatograms, of plasma samples containing lambda cyhalothrin.

Peak number: (1) LCT (1R, cis, S); (2) LCT (1S, cis, R); (3) internal standard (EI) (PCB -166).

3.4 Histopathological findings

3.4.1 Testes

Testicular sections of the control healthy rats had normal histo-architecture that consisted of uniform, well-organized seminiferous tubules with complete spermatogenesis and normal interstitial connective tissue (Fig. 2A). Testicular tissue of rats received three doses of LCT showed degenerative changes of the majority of the seminiferous tubules (Fig. 2B, 1C, 1D). These changes were characterized by disorganization and damaged seminiferous tubule which showed complete spermatogenic arrest, less lumen formation contained cellular debris and less interstitial cells or Sertoli cells in the testis of the rat treated with LCT. Regarding to the interstitium, there were congestion of the interstitial blood vessels and edema that was represented by faint eosinophilic material.

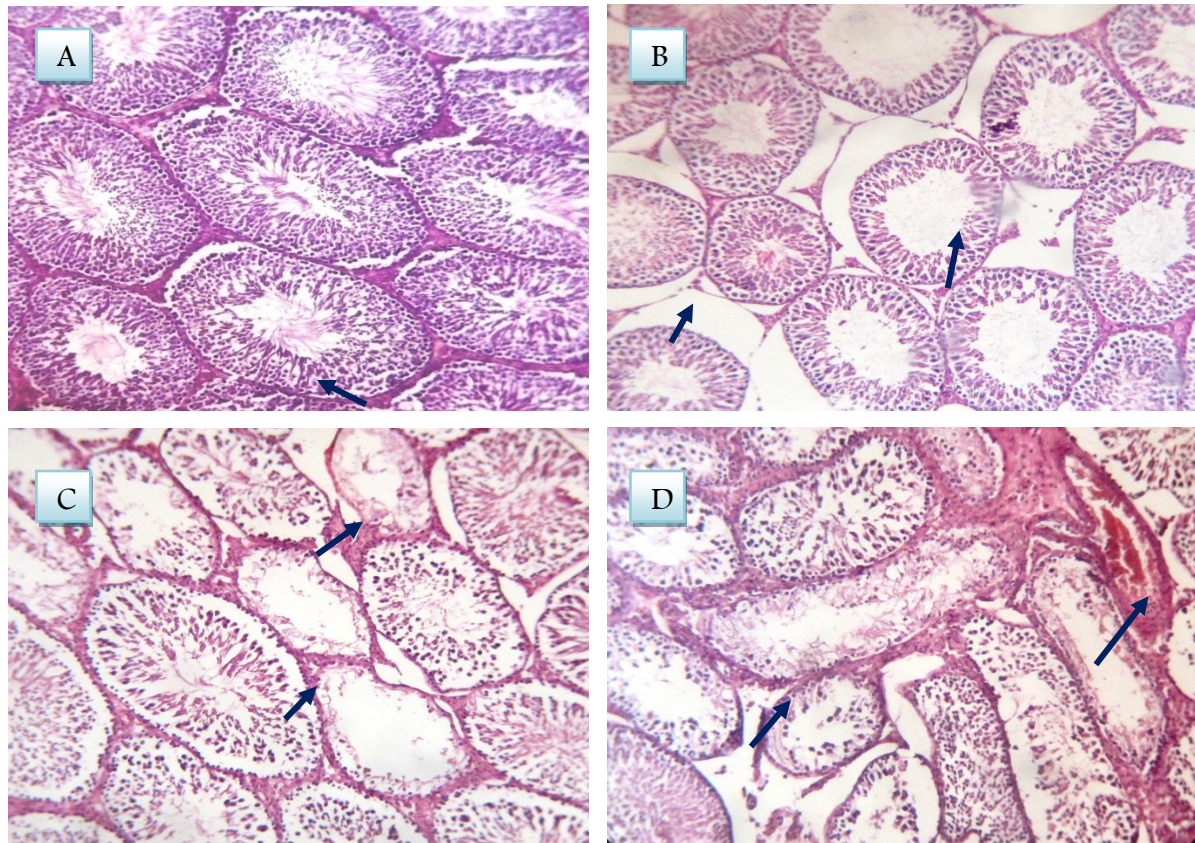


Figure 2. Photomicrograph of rat testes stained with HE x 200: (A) Testis of a control rat showing normal testis histo-architecture of mature seminiferous tubules, associated with complete spermatogenic series (arrows). (B) Testis of rat given 20.4 mg of LCT /kg b.w. Showing disorganized seminiferous tubules (arrows). (C) Testis of a rat given, 30.6 LCT mg /kg b.w. Showing degeneration and necrotic of germ cell lining seminiferous tubule (arrows), vacuolation (arrows). (D). Testis of a rat given 61.2 mg of LCT /kg b.w. showing congestion (arrows), complete spermatogenic arrest (arrows).

3.4.2 Seminal vesicle

Seminal vesicle of the control rats had no obvious histopathological alterations (Fig. 3A). Seminal vesiculitis was evident in the LCT treated rats, atypical hyperplasia and degeneration of the seminal vesicles was found in the high dose (61.2 mg/kg) LCT treated rats when compared with that of controls.

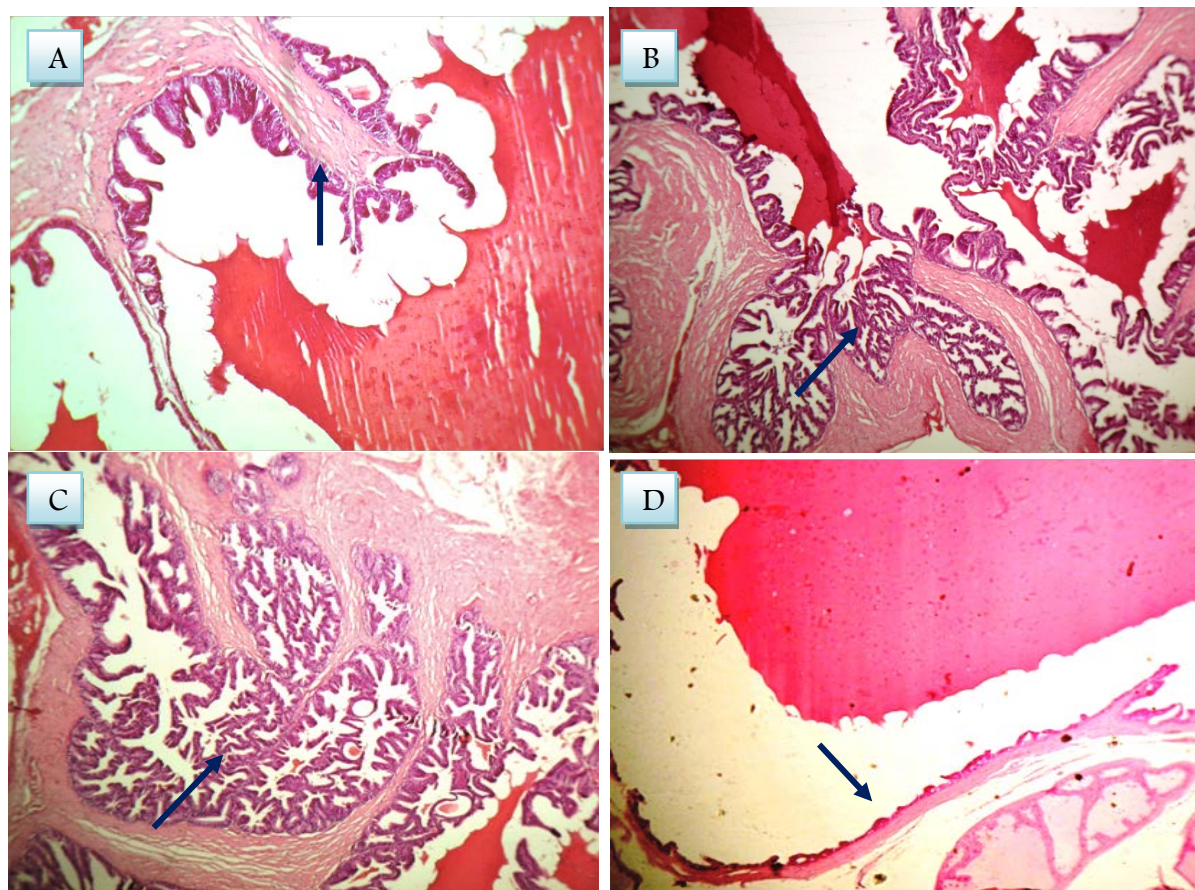


Figure 3. Photomicrograph of rat seminal vesicle stained with HE x 200: (A) Seminal vesicle of the control rats with normal structure (arrows). (B) Seminal vesicle of rat given 20.4 mg of LCT /kg b.w. Showing atypical hyperplasia (arrows). (C) Seminal vesicle of a rat given, 30.6 LCT mg /kg b.w. Showing atypical hyperplasia (arrows). (D) Seminal vesicle of a rat given 61.2 mg of LCT /kg b.w. showing degeneration of the epithelium (arrows).

4. DISCUSSION

In the present work, mean daily food consumption and body weight gain were significantly reduced in rats gavaged with middle and high dose of lambda cyhalothrin (LCT) during 28 days of dosing. The decline in the body weight of treated rats with LCT appeared as a result of lesser intake of feed (Table 1).

Table 2 shows that absolute and relative weight of testes and seminal vesicles were reduced by lambda-cyhalothrin treatment. These findings were in agreement with those of Youcef *et al.* (2003) who reported cytotoxic effects of the pyrethroid insecticide on male rats which exhibited a significant decrease in weights of testis, epididymis, seminal vesicle and prostate glands. Also, El-Demerdash (2007), showed significant reduction in the index weight of testes of exposed animals following exposure to cypermethrin or other closely related type-II pyrethroids.

The results of the present study revealed that oral administration of lambda cyhalothrin at a dose of 20.4, 30.6 and 61.2 mg/kg b.w. for 28 successive days to male Wistar rats induced deleterious effects on organs of male fertility as elucidated by the significant decrease in seminal vesicles and testicular index weights. The observed decrease in organ index weights of male rats in this study may be attributed partly to the direct cytotoxic action of the insecticide in these tissues. Such decrease could also be referred to the reduced testosterone synthesis and disruption of normal androgen status (Hossain and Richardson, 2011).

Oral ingestion of LCT, significantly decreased the count of RBCs, Hb and MCV content of intoxicated rat relative to control (Table 3). This probably explained by the effect of lambda cyhalothrin on the erythropoiesis and the destruction of cells (Dahamna *et al.*, 2009).

In the current study, at day 28 residual concentrations of the two isomers of LCT (1R, cis, S and 1S, cis, R) were found in all plasma of rats treated with 30.6 mg/kg and 61.2 mg/kg of LCT in dose dependant manner, and two peaks were observed in the chromatograms for lambda cyhalothrin due to the separation of its diastereoisomers (Liu and Pleil, 2002). However, a handicap in analyzing LCT is that the concentration of each isomer in the standard mixture should be known but, generally, the technical standard mixtures of pyrethroids only indicate the sum of the concentrations of the individual isomers for each pyrethroid (Li *et al.*, 2014).

Synthetic pyrethroids have been considered potentially toxic to male reproductive system (Kilian *et al.*, 2007); they have ability to disrupt estrogen signaling and affect male reproductive organs and semen quality. Exposure to pyrethroids causes decreased sperm counts, impairment of sperm motility, reduced fertilization ability, producing abnormal sperm in the rodents following repeated exposure (Wang *et al.*, 2014).

Histopathological examination of the seminal vesicle (Figs. 3 A–D) of rats given LCT at 20.4, 30.6 and 61.2 mg/kg b.w. showed atypical hyperplasia and degeneration of the epithelium. In addition the examined testes of LCT-treated rats showed marked degeneration of spermatogenic cells associated with interstitial necrosis of spermatogenic cells and congestion with interstitial diffuse edema. These results are in agreement with the previous studies in rat showing sexual dysfunction of male reproductive organs, reduction in testosterone levels after pyrethroid treatment (Ratnasooriya *et al.*, 2002; Yousef *et al.*, 2003; Yousef, 2010; Hossain and Richardson, 2011). Additionally, Yu *et al.*, (2014) found that treatment with deltamethrin inhibited sperm production and increased germ cells apoptosis which caused reduction in the fertility of male rats (Issam *et al.*, 2009). The observed Histopathological results, in lambda cyhalothrin-treated animals, might suggest that this chemical exposure causes deterioration of germinal epithelium and seminal vesicle epithelium. In fact, LCT may influence cell damage due to its plasma membrane lipophilicity (Issam *et al.*, 2009).

CONCLUSION

The present data demonstrate the detrimental effect of lambda cyhalothrin on male reproductive organs (testes and seminal vesicles), plasma residue and hematological parameters in male rats following repeated exposure to this insecticide.

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