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Testicular Toxicity of Cypermethrin, Lambda Cyhalothrin and Deltamethrin- A Comparative Histopathological Study

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ABSTRACT

Purpose: The present study was designed to explore the comparative toxicological implications of three halogenated type II pyrethroid insecticides that are cypermethrin, deltamethrin and lambda cyhalothrin on testis of adult Swiss Webster male albino mice (Mus musculus).

Methods: The animals (40) of almost similar body weight and age were randomly distributed in four groups (N=10) that are; 1: Control group treated with vehicle that is corn oil for three days, 2: Cypermethrin group treated with 0.6 mg/kg body weight for three days using corn oil as vehicle, 3: Deltamethrin and 4: Lambda cyhalothrin groups treated with respective insecticides as in group no 2 that is cypermethrin group. The testes were recovered on the day seven and processed for wax embedded microtomy and Hematoxylin & Eosin (H & E) staining.

Results: The results have shown various testicular pathologies in the three insecticide groups. Cypermethrin had shown only one whirl of spermatogonia and few spermatocytes along the basement membrane, while in deltamethrin group the seminiferous tubular sections were having few spermatogonia and spermatocytes as compared to the control and cypermethrin group. Similarly various significant alterations in micrometry as decreasing seminiferous tubular count per 850 µ2 unit area was observed in all the insecticide groups as compared to the control group.

Conclusions: The finding among three insecticides suggests that the fluorinated pyrethroid lambda cyhalothrin was most toxic among the three pyrethroids then the brominated pyrethroid deltamethrin. Lambda cyhalothrin may inflict more deleterious effects on the testicular anatomy as compared to the deltamethrin.

Keywords: *Cypermethrin; Lambda cyhalothrin; Deltamethrin, Testicular toxicity*

INTRODUCTION

Pesticides can be divided into several chemical families; comprising the pyrethroids, organo-chlorine complexes, carbamates, organo-phosphorus mixes, triazines combinations and neonicotinoids.[1] In agriculture, industry and medicine, the organophosphorus compounds were mostly used; but now pyrethroids are replacing them because of their photo-stability, rapid breakdown, effectiveness in low concentration and low toxicity in birds and mammals.[2] Cypermethrin, deltamethrin and lambda cyhalothrin are synthetic pyrethroids; comprehensively used in agricultural sector and civic health for prevention and regulation of pests and insects.[3] Cypermethrin is a hydrophobic compound which can easily cross cell membrane and disturbs its structure and causes the leakage of cytoplasmic content.[4] Deltamethrin is readily absorbed in contaminated water and food[5] and has high bioavailability in feces and urine.[6] Lambda cyhalothrin is found in blood and milk of dairy cows[7] and in meat of cattle.[8]

The cypermethrin not only decreases the amount of leydig cells, sertoli cells and germ cells; but it also reduces the count of cell layers in the seminiferous tubules. It causes the vacuolization in sertoli cells and deformed nucleus in leydig cells. In serum, the concentration of testosterone lowers significantly. It suggests that cypermethrin might decreases spermatogenesis by decreasing the testosterone production.[9]

Deltamethrin lowers the body weight and the quantity of seminiferous tubules in 850 μ^2 unit area and increases the weight of testis. Moreover, it decreases diameter of seminiferous tubules.[10] Lambda cyhalothrin causes sexual dysfunction in male rats by toxicity and stress.[11] Studies have shown that there is a need to know the toxic effects of halogenated type II pyrethroid insecticides like, cypermethrin, deltamethrin and lambda cyhalothrin to investigate their impact on non-target animals like human. This whole research work has been planned to evaluate the toxic effects of such chemicals in albino mice.

MATERIAL AND METHODS

2.1 Animal maintenance:

The present experiment was designed for 40 adult three months old male Swiss Webster mice (*Mus musculus*); weighed 25-25g with body length 7-10cm and tail length 5-10cm. They were kept in animal house of UOS (University of Sargodha). Before starting the experiment, the animals were allowed to adjust themselves to new environment for 7 days. They were kept in plastic cages having stainless steel on inner side. They were provided with fresh water and feed and non-toxic printing papers for bedding; which were changed on daily basis, the debris was also removed every day. They were kept in 12-12 hour's dark and light round. The temperature was established at 24±2°C and humidity at 45%.

2.2 Experimental Design:

40 animals were divided in 4 groups having 10 animals in each group. These groups were named as Control (Cnt), Cypermethrin (Cyp), Deltamethrin (Del) and Lambda cyhalothrin (Lct).

2.3 Preparation of Cyp, Del and Lct solutions:

The technical grades of viscous liquid Cyp (94%), crystalline Del (98%) and solid Lct (97%) were purchased from online retailers Kissan Ghar (https://kissanghar.pk). The stock solution of 20 mg/kg was prepared and it was further diluted to 5mg/kg to get the desire concentration.

2.3.1 Preparation of stock solution of 20mg/kg:

20mg/kg connotates

Required dose for animal of 1000 gram = 20mg

Requisite dose for animal of 1g = 20mg/1000g

Required dosage for animal of 30g = 20mg/1000g $*$ 30g = 0.6mg/30g

Each animal received 50 micro litres of Cyp, Del and Lct. It means that 50 micro litre of 20mg/kg Cyp, Del and Lct solution should contain 0.6 mg of Cyp, Del and Lct.[12]

2.3.2 Dose Preparation:

The following formula was used to prepare the required dose;

$$Concentration\ of\ primary\ fluid\ X\ Volume\ of\ primary\ fluid = Concentration\ of\ ultimate\ blend\ X\ Volume\ of\ ultimate\ blend$$

$$\mathbf{C1V1 = C2V2}^{13}$$

2.4 Experimental groups:

Division of different groups (i.e., Control group, Lct group, Del group and Cyp group) was based on given specific pesticide solution.

2.5 Dose Administration:

All drugs were orally administered to different groups of animals. Throughout the study, the standard volume of dose was 0.1mL.

2.5.1 Control Group *(Cnt):* It received 0.1mL/24 hours of corn oil for 6 days through gavage.

2.5.2 Lambda cyhalothrin Group *(LCT):* It received 0.1mL/24 hours of 5mg/kg lambda cyhalothrin solution in corn oil through gavage on day 1,2 and 3 followed by resting period of 3 days.

2.5.3 Deltamethrin Group *(DEL):* It received 0.1 mL/24 hours of 5mg/kg deltamethrin solution in corn oil through gavage on day 1, 2 and 3 followed by resting period of 3 days.

2.5.4 Cypermethrin Group *(Cyp):* It received 0.1 mL/24 hours of 5mg/kg cypermethrin solution in corn oil through gavage on day 1, 2 and 3 followed by resting period of 3 days.

2.6 Recovery Period and Daily Observation:

Animals were kept for recovery period of 3 days after the application of particular dose. In recovery period, the animals were given proper food and water. Before feeding, the body weight was measured and checked if there is any variation. To measure the weight, digital weight balance of 0.1g precision was used.

2.7 Dissection and organ recovery:

At day 7, the animals were dissected. The weight of each animal was noted before dissection. By cervical dislocation, the animals were euthanized and with the help of forceps and scissors, the belly was opened to expose the organs.

Both testes were removed and immersed in saline water. Then both were placed in Carnoy's fixative to solidify them for sectioning and to protect them from autolysis.[14]

2.8 Testicular histology:

After keeping the organ in fixative for 48 hours, organs were preceded for wax embedding. It includes immersing of organ in 50%, 70%, 90% and 100% ethanol for dehydration. This is followed by clearance in xylene.

Blocks of testis were prepared; then sections were obtained at 3-4µm by using rotary microtome (ERMA TOKYO 422). Sections were stretched at 45°C in water bath and were attached on glass slides treated with albumin. Slides were formerly stained by means of hematoxylin and eosin for micrometric and histopathological studies.[14]

2.9 Testicular Observations:

Testicular sections were then cautiously observed on microscope (Labomed CXR2) and photographed at 100x and 400x with digital camera (Sony DSC-W55) of 7.2 mega pixel resolution.

2.10 High-tech processing of digital micro-snapshots:

To highlight the pathological influences, the selected snaps of testicular sections were upgraded in CorelDraw for colors, shade, cropping, contrast and for emphasizing the signs and then demonstrated in the result section.

2.11 Micrometry and statistical application:

For micrometry, ten testicular sections were randomly selected from entire animals of four groups and were snapped at 100x and 400x magnifications. To measure the cross-sectional area (CSA), length and width were measured by using Bezier tool. The standardized values were put in the following formulas.

CSA (of seminiferous tubules and spermatogonia)

= (Length Width/4) π

The micrometric data was analyzed by applying to the ANOVA and Tukey multiple Range test (TMRT) in IBM SPSS-20 Software.

RESULTS

3.1 Histopathological results:

The histology of control group animals shows the rounded cross sections of seminiferous tubules; containing concentric arrangements of spermatogonia, spermatocytes, spermatids and maturing spermatozoa from margin to the centre of each tubular section. The spaces between the tubules were thickly populated by the interstitial cells that are endocrine in nature and produce male sex hormones; the androgens **(Fig: 1A, 2A)**.

In cypermethrin treated group, the number of whirls of spermatogonia along the basement membrane was restricted to one; and with only few whirls of spermatocytes. The wide lumens of seminiferous tubules were shown to contain spermatocytes, spermatids and maturing sperms. The interstitial tissue showed scanty appearance of leydig cells indicating interstitial tissue's cell death **(Fig: 1B, 2B)**.

In testicular sections of lambda cyhalothrin, the interstitial tissue was shrunken with fluid filled empty spaces present in the lieu of interstitial cells. The seminiferous tubular sections were enormously enlarged; showing wide empty spaces in the centre, containing only few spermatozoa. The spermatogonia along the basement membrane were also distributed with wide spaces in between them. The spermatocytes also showed wide spaces around them **(Fig: 1C, 2C)**.

In deltamethrin group, the seminiferous tubular sections were having far lesser number of spermatogonia along the basement membrane as compared to the control and the cypermethrin groups. The spermatocytes showed vacuolations. They were also far scanty in number; the central lumen was almost completely empty, containing only a few dislocated sperms. The interstitial tissue contained comparatively higher number of cells as compared to lambda cyhalothrin group, however; the empty spaces around the seminiferous tubules were created due to interstitial tissue's cells death **(Fig: 1D, 2D)**.

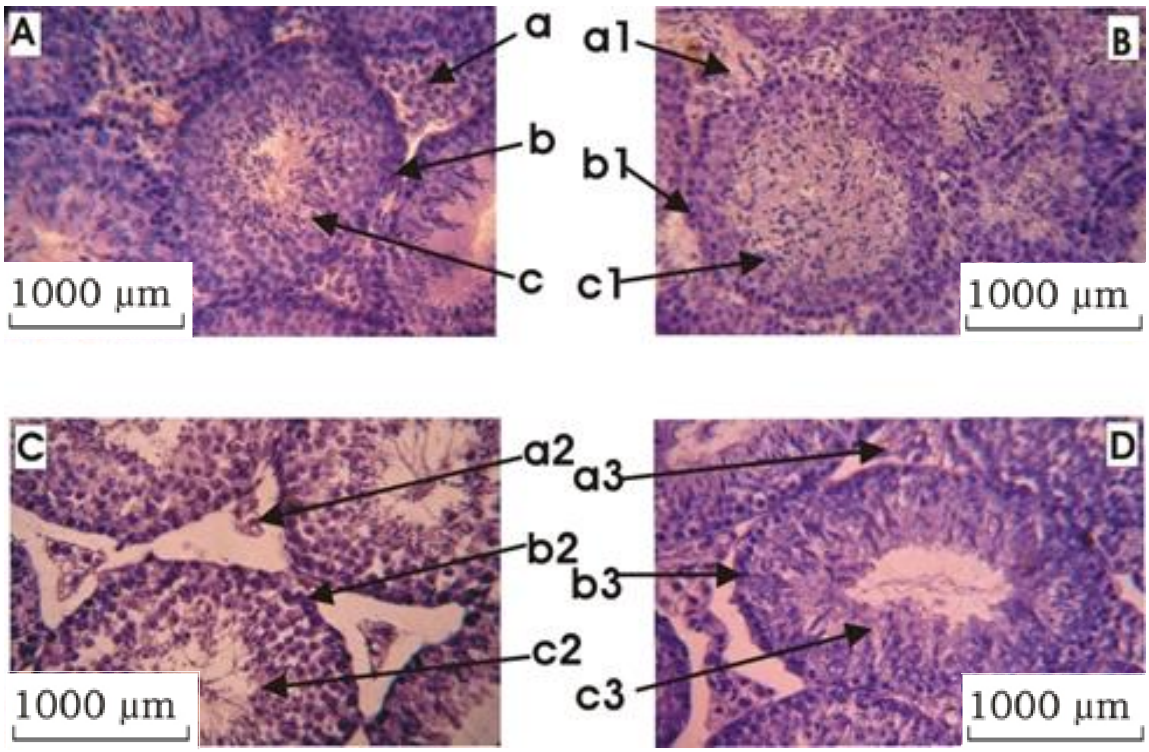

Figure 1. Digitally calibrated scale (1000 µm) stated on the photomicrographs. Hematoxylin and Eosin-stained histological sections (400x) of mice testes. **A**: (Con); **B**: (Cyp); **C**: (Lct); **D**: (Del), **a:** Healthy Interstitial tissue **a1, a2, a3:** damaged interstitial tissue **b:** healthy seminiferous tubule **b1, b2, b3:** damaged seminiferous tubules **c:** healthy spermatids **c1, c2, c3:** damaged spermatids.

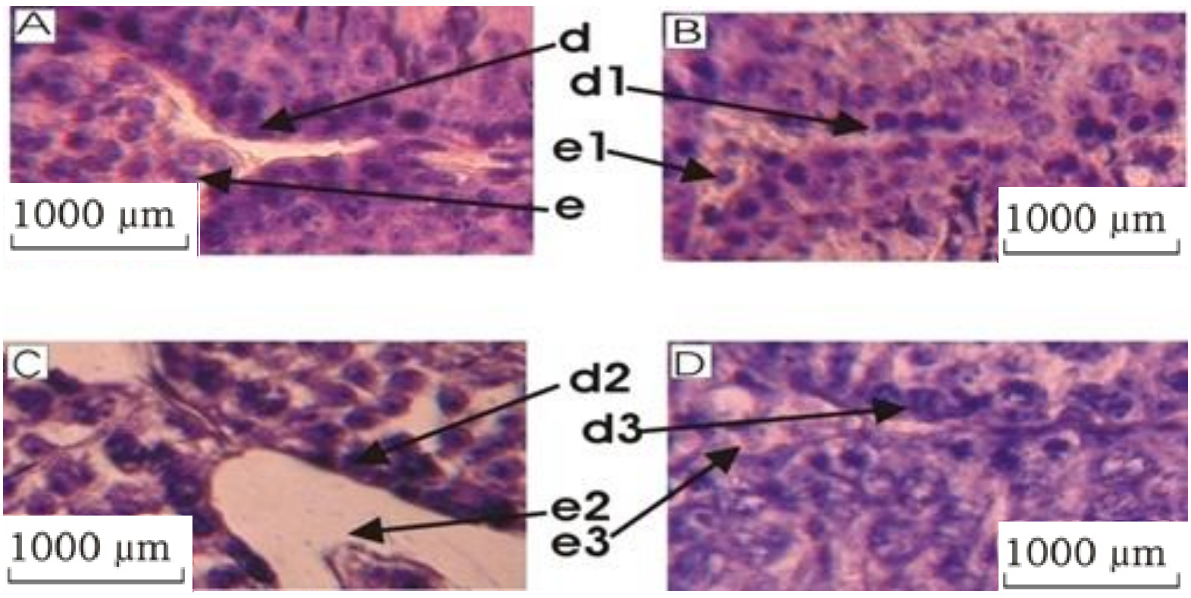

Figure 2. Digitally calibrated scale (1000 µm) stated on the photomicrographs. Hematoxylin and Eosin stained histological sections (400x) of mice testes. **A**: (Con); **B**: (Cyp); **C**: (Lct); **D**: (Del), **d:** Evenly arranged and thickly populated spermatogonial cells **d1, d2, d3:** necrosis of spermatogonial cells **e:** interstitial space having interstitial cells **e1, e2, e3:** interstitial cell death.

3.2 Micrometric results:

3.2.1 Mean CSA of seminiferous tubules:

The sum total means of cross-sectional area of seminiferous tubules in control group endured significantly lesser (13776.6±592) than all the three insecticide treated groups. However, the highest midpoint cross sectional area of seminiferous tubules was recorded in lambda cyhalothrin (30173.8±775.0) followed by deltamethrin (27809.2±927.6), which was even significantly higher than the mean cross-sectional area of the cypermethrin group (19523.1±582.4) (Table. 1).

3.2.2 Mean CSA of spermatogonia:

The CSA of spermatogonial cells was enormously enlarged in lambda cyhalothrin group (82.6±4.0 c) and differs very highly significantly with the rest of three groups. However, the deltamethrin (63.3±1.9) and the cypermethrin (52.6±2.5) groups also showed significantly higher mean cross-sectional area of spermatogonia as compared to the control group (36.7±3.4) (Table 1).

3.2.3 *Number of spermatogonia per unit circumference of seminiferous tubule:*

Statistical analysis of data using one-way ANOVA for mean number of spermatogonia per unit circumference of seminiferous tubule showed the highly significant (p≤0.0001) alteration amid the groups. The Lambda cyhalothrin showed the least number of spermatogonial cells (9.3750±0.28) as compared to Deltamethrin (9.2750±0.35) and cypermethrin (10.55±0.30) groups. While control group (11.95±0.27) showed the highest number of spermatogonial cells per unit area (Table 1).

3.2.4 Mean CSA of leydig cells:

Although the mean cross-sectional area of interstitial cells in cypermethrin (26.4±1.2) and deltamethrin (30.8±3.0) groups were not significantly different to that of the control group (25.8±1.3); but a considerable increase in cross sectional area was noted to that of the control group. Once again in this parameter, the mean cross-sectional area of lambda cyhalothrin group (33.3±1.6) remained significantly higher than the control group (25.8±1.3) (Table 1).

3.2.5 Mean number of seminiferous tubules in 850µm x 850µm area:

The quantity of seminiferous tubules per unit area that is 850µm^2 was expressively higher in control group (12.7±0.27) than rest of the three groups; whereas the lambda cyhalothrin group has shown the least numerals of seminiferous tubules per unit area (3.6±0.40); leaving the deltamethrin (4.9±0.13) and the cypermethrin groups (7.0±0.19) in between. However, all the three insecticide treated groups showed significant variations with each other as well as with the control group (Table 1).

Table 1. Micrometric results of various parameters of testis (CSA= cross sectional area)

Histometric/micrometric parameters	Mean±SEM			
	Control	Cypermethrin	Deltamethrin	Lambdacyhalothrin
Mean CSA of seminiferous tubules (µm^2)***	13776.6±592a	19523.1±582.4b	27809.2±927.6c	30173.8±775.0c
Mean CSA of spermatogonia (µm^2)***	36.7±3.4a	52.6±2.5b	63.3±1.9b	82.6±4.0c
Number of spermatogonia per unit circumference of seminiferous tubule (50µm)***	11.95±0.27c	10.55±0.30b	9.2750±0.35a	8.3750±0.28a
Mean CSA of leydig cells (µm^2)*	25.8±1.3a	26.4±1.2ab	30.8±3.0ab	33.3±1.6b
Number of seminiferous tubules per unit area (850µm^2)***	12.7±0.27d	7.0±0.19c	4.9±0.13b	3.6±0.40a

ł: analyzed by ANOVA, abc the mean values in a row not inputting a conjoint superscript vary ominously (p≤0.05) with each other.

DISCUSSION

In the present study, the relative testicular histopathology and micrometry of three type II pyrethroid insecticides were compared keeping in view of presence of various specific halogens. The Cypermethrin is simply chlorinated; whereas the Lambda cyhalothrin contains fluoride ions and the Deltamethrin is brominated insecticide.

Fluorine and bromine being the non-dietary elements have been found to inflict specific toxic effects on the exposed animals; whenever they have been used in the form of their ions like fluoride exposure[15] There are various indications that cypermethrin has been causing various toxicological and histopathological effects on nervous tissues, cardiac tissues and hepatic tissues and organs of the exposed animals like rats, rabbits and mice.[16]

Whereas, the deltamethrin is also found to cause various health effects and organ toxicities; which included hepatotoxicity, neurotoxicity and testicular toxicity in rabbit, mice, rats and pig. Unfortunately, there is very little data available pertaining to the organ toxicology of lambda cyhalothrin, the fluorinated insecticide of the type II pyrethroid groups used in the present research work.

The fallouts of the present inquiry have shown the interesting toxicological implications of the three insecticides used; for example, the cypermethrin has been found to lower the number of spermatogenic cells **(Fig: 2B, C & D; Table: 1)**, increase the inner diameter of seminiferous tubules, lower the number of sperms in the testis, cause the hepatotoxicity and neurotoxicity by crossing the blood brain barrier of the exposed animal **(Fig: 1B, C & D; Table: 1)**.

In addition to these effects, the deltamethrin has been found to cause excessive salivation, impaired limb function, ataxia, lethality, and rarely paralysis in rabbit, mice, rats and pig through skin, mouth and inhalational pathways.[17, 18] Whereas; the lambda cyhalothrin exposure was found to lead to the abnormalities related to reproduction, cancer and nervous system.[19]

The micrometric estimations have also revealed significant changes in the CSA of seminiferous tubules, spermatogonial cells, and leydig cells, and the number of seminiferous tubules and spermatogonia per unit area **(Table: 1)**. The forth going discussion indicates that the exposure effects of cypermethrin, lambda cyhalothrin and deltamethrin noted in the present exploration are mostly consolidating and in line with the previously available research papers.[20-23]

The results of present study showed that lambda cyhalothrin has inflicted more severe testicular damage than that of cypermethrin and deltamethrin; indicating the higher susceptibility of mammalian testis towards fluorinated pyrethroids; the lambda cyhalothrin **(Fig:1C; Table: 1)**. It is thus suggested that the pyrethroids (especially halogenated) must be used with the great caution and care because their sustained exposure may lead to various testicular damages; ultimately affecting the process of spermatogenesis, leading to male infertility.

CONCLUSIONS

The type II pyrethroids are likely the disruptor of spermatogenic action and sperm viability in testes and can produce remarkably distinctive histopathological fluctuations, i.e., non-flourinated pyrethroid insecticides CYP caused the fluid accumulation predominantly in the interstitial tissue. The fluorinated insecticide LCT caused the more accumulative and persistent toxicological influence of LCT than that of non-fluorinated CYP and DEL. It is thus concluded that LCT has more potential as reproductive toxicant as compared to other selected insecticides of the pyrethroid group.

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ETHICAL STATEMENT

This research has been evaluated and sanctioned by the Ethics Committee and Research Board, “Department Of Zoology”, “University Of Sargodha” (SU/Zol/295) and meets the ethical standards requisite for project involving human/animal subjects (i.e., mice).

CONFLICT OF INTERESTS

“None”.

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