IJAAR 4 (2016) 38-45 ISSN 2053-1265

International Journal of Advance Agricultural Research
www.bluepenjournals.org/ijaar

Sub-chronic toxicity study of synthetic Pyrethroids (Lambda-cyhalothrin) on reproductive organs of male Wistar rats

Oshoke F. I., Fadina, O. O.* and Fayinminnu, O. O.

Department of Crop Protection and Environmental Biology, Faculty of Agriculture and Forestry, University of Ibadan, Ibadan, Nigeria.

Article History
Received 18 April, 2016
Received in revised form 09 May, 2016
Accepted 17 May, 2016

Keywords:
Pyrethroid, Toxicity, *Rattus norvegicus*, Testicular tests, Gavage.

Article Type:
Full Length Research Article

ABSTRACT

Pesticides constitute the most widespread environmental contaminants due to their ubiquitous use in all aspect of human endeavors. Lambda-cyhalothrin (LTC), a synthetic pyrethroid has widely been used for pest control. Human and animals are occasionally and unintentionally exposed to lethal and sub-lethal doses of pesticides stemming from its various uses to control pests both in agriculture and public health. The objective of the study was to evaluate the toxicity of LTC in male reproductivity through testicular tests. The study also investigated the haematological, serum biochemistry and histological effects of sub-chronic concentrations of LTC on male rats. Twenty-five 7-weeks-old male Wistar rats (*Rattus norvegicus)* were randomly assigned to five groups. Group 1 was the control group, which received distilled water. Experimental groups 2, 3, 4 and 5 received by gavage 25, 50, 75 and 100 mg/kg LTC body weight, respectively, of LTC over a period of five weeks. Histopathological studies were carried out on the testes and seminal vesicles at the end of the experiment. A significant decrease in the absolute weight of testes and seminal vesicles, sperm count, sperm motility and L-D ratio was observed. The results obtained also show marked degeneration of spermatogenic cells associated with interstitial necrosis and congestion with interstitial diffuse edema in the testis of the rat treated with LTC. A typical dose-dependent hyperplasia and degeneration of the seminal vesicles was found in all LTC treated rats with 100 mg/kg body weight and 25 mg/kg body weight having the highest and lowest toxicity level respectively, when compared with that of Group 1. It can be concluded that LTC is highly toxic and may induce poor fertility, cell damage and anaemic conditions in exposed rats.

INTRODUCTION

Pesticides are killers of pests. Lambda-cyhalothrin (LTC), a synthetic pyrethroid has been widely used for pest control in agriculture, public health, homes and gardens to control a wide range of Lepidotera, Hemiptera, Foptera and Coleoptera species of insects. The use of pyrethroid products has grown and would continue to grow due to the suspension of some organophosphorous and organo chlorines. Pesticide exposure is associated with long-term health problems such as respiratory problems, memolambda-cyhalothrinry disorders, dermatologic conditions, cancer, depression, neurological deficits, miscarriages and birth defects (Gupte et al., 1991).

In a bid to make food available to the teeming world population, there has been an increased global drive for

*Corresponding author. E-mail: bumiofad@yahoo.com.

commercialization, mechanization and large scale production of agricultural produce. This has led to unprecedented outbreak of pests, disease incidence and prevalence (Oros and Werner, 2005). This emergency situation promptly ushered in the era of synthetic pesticide administration in agriculture since pesticides are known to be achieving rapid knockdown effect on target pests, but not without consequent adverse effect on non-target organisms.

Synthetic pyrethroid insecticides are structural analogue of pyrethrins which are insecticidal compounds obtained from plants such as *Chrysanthemum cinerariaefolium* and *Chrysanthemum cineum* (Thatheyus and Salvam, 2013).

LCT is a synthetic pyrethroid insecticide and acaricide used to control a wide range of pests in a variety of application. It was developed in 1977 (Lee et al., 1998). It has a high level of activity against a wide range of Lepidoptera, Hemiptera, Diptera and Coleoptera spp. of insects. It also has miticidal activity (Southwood, 1985). LTC is a stomach, contact and residual insecticide (Southwood, 1985).

LTC ($C_{22}H_{19}ClF_3NO_3$) 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). It has a reported LD_{50} of 55–80 mg/kg body weight in rats treated with an oral dose of cyhalothrin in corn oil as reported by Southwood (1985).

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).

This current study was performed to assess the toxicity of LTC on reproductive organs, testes and seminal vesicle, of male fertility during sub-chronic LTC exposure and to further investigate the dose effect of LTC on hematological parameters.

MATERIALS AND METHODS

A total of 25 individual of *Rattus norveigicus* aged 3-4 weeks old with weight range 100–150 g were used for this study. Wistar rats were procured from the Animal House of the Department of Physiology, College of Medicine, University of Ibadan, Nigeria. The rats were acclimatized for three weeks before the commencement of the experiment. The rats were fed on growers mash throughout the duration of the study and the feed was purchased from a local agroshop. Water and feed were supplied *ad libitum*.

Lambda-cyhalothrin

The pesticide (Kombat) was purchased at an agro chemical shop (AMENNS GROUP) in Ibadan, Oyo State, Nigeria. All other chemicals required for biochemical, haematological and histopathological estimation were of high quality and were provided by the Department of Pathology, Veterinary Medicine, University of Ibadan.

The pesticide LTC was mixed with a vehicle (water). The concentration of the active ingredient in *Kombat* is 25 g/L (on label), this is also equivalent to 25,000 mg/L. 2 mls of the pesticide solution was taken and distilled water added to make it up to 500 mL, this gave a solution of the active ingredient in 1,000 ppm. These concentrations were determined using the formula:

$$C_1V_1 = C_2V_2$$

Where;
C_1 = initial concentration of stock
V_1 = volume of stock used
C_2 = desired concentration
V_2 = desired volume

From this stock solution, the following treatments were taken and distilled water added to make it up to 250 mL.

Treatment 4: 6.25 mL equivalent to 25 mg/L
Treatment 3: 12.5 mL equivalent to 50 mg/L
Treatment 2: 18.75 mL equivalent to 75 mg/L
Treatment 1: 25 mL equivalent to 100 mg/L

Experimental set up/toxicological study

The experiment was laid out in a completely randomized design (CRD) with 5 treatments replicated 5 times. The Cage used for the experimental animals was constructed with feeders and drinkers and properly placLambda-cyhalothrined in a well ventilated area of the second year laboratory, Department of Crop Protection and Environmental Biology. The 25 Wistar rats were divided into 5 groups of 5 rats each.

The various levels of the pesticide formed the treatment with a control group (no pesticide). The treatment levels were:

T4= 25 mg/kg of LCT
T3= 50 mg/kg of LCT
T2= 75 mg/kg of LCT
T1= 100 mg/kg of LCT

All doses (treatments) were administered daily (in the mornings) by oral gavage (with syringe and cannula) for a period of five weeks. Animals were weighed weekly. At the end of the experiment, animals were sacrificed by cervical dislocations. The organs of interest (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:

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

Data collected

The animals were weighed weekly in order to determine weight gain/loss also; the feed given was weighed in order to ascertain the daily feed intake.

Physical observations such as shyness, behavioral pattern of the rats, signs of pesticide toxicity such as tremors, loss of appetite, muscle jerking, constricted pupils, vomiting etc. (Fadina et al., 1999) were recorded per animal in each treatment.

Histopathological analysis

On termination of the experiment, all the surviving Wistar rats were sacrificed and organs of interest (testes and seminal vesicles) were harvested via cervical dislocation. These organs included the testes and seminal vesicles of each rat. In preparing the animal tissues (testes and seminal vesicles) of each rats for microscopic examination, histological procedures were followed in a stepwise protocol thus: fixation, dehydration, clearing, infiltration, embedding, blocking, sectioning and staining (Hayat, 1989; Bancroft et al. 2002 and Avwioro, 2010).

Reproductive fertility evaluation

Spermatozoa were obtained by mincing the cauda epididymus in a known volume of physiological saline (w/v) at 37°C for evaluation of semen parameter under microscope (×400) as following: Sperm concentration count expressed as sperm concentration in millions according to Feustan et al. (1989); Sperm motility according to Linder et al. (1986); Sperm viability by Eosin stain according to Krzanowska et al. (1995).

Statistical analyses

Data obtained from the study were subjected to one-way analysis of variance ANOVA (SAS Version 9.2, 2002-2003) while group means were compared for significant differences at 95% confidence level ($P<0.05$) (Duncan, 1955). Data obtained from testicular reproductive parameters were statistically analyzed using student's *t*-test. $P<0.05$ was considered significant (Petrie and Watson, 1999). The results are presented as mean± standard error of the mean (SEM).

RESULTS

Behavioral/morphological responses

All Wistar rats in the experimental set up were found to exhibit shyness during gavage sessions. All the rats were observed to rub their lip region and face with their fore limb after treatment administration. General loss of appetite was also noticed in all treated rats especially amongst rats administered with the highest concentration of LTC (100 mg/kg).

Obvious behavioral response to pesticide toxicity was evident amongst most of the LTC treated rats. This included nasal discharge, mucus sealed eyes and paralyzes. All rats that exhibited signs of paralyses died before the termination of the experiment.

A significant decrease in body weight and average feed intake were observed for rats treated with LTC when compared with control rats (Figure 1).

Reproductive study

The results obtained with respect to sperm count of LTC treated rats show that there was a progressive significant decrease ($p<0.05$) as the concentrations of LTC increased (dose dependent) when compared with the control (Figure 2). Sperm motility value was significantly decreased ($p<0.05$) in rats treated with LTC (Figure 3). Also, on percentage abnormal sperm cell values and live dead (L-D) ratio of treated rats significantly elevated and decreased respectively when compared to control which recorded the highest (Table 1). Similarly, a significant decrease in the absolute and relative weights of seminal vesicle and right and left testes was observed (Table 2).

Histopathological examination of the testes of LTC-treated rats showed marked degeneration of spermatogenic cells associated with interstitial necrosis and congestion with interstitial diffuse edema (Figure 4). There was no obvious histopathological alteration in the Seminal vesicle of the control rats. Seminal vesiculitis was evident in the LTC treated rats, a typical degeneration of the seminal vesicles was found in 100 and 75 mg/kg of treated rats when compared with that of control.

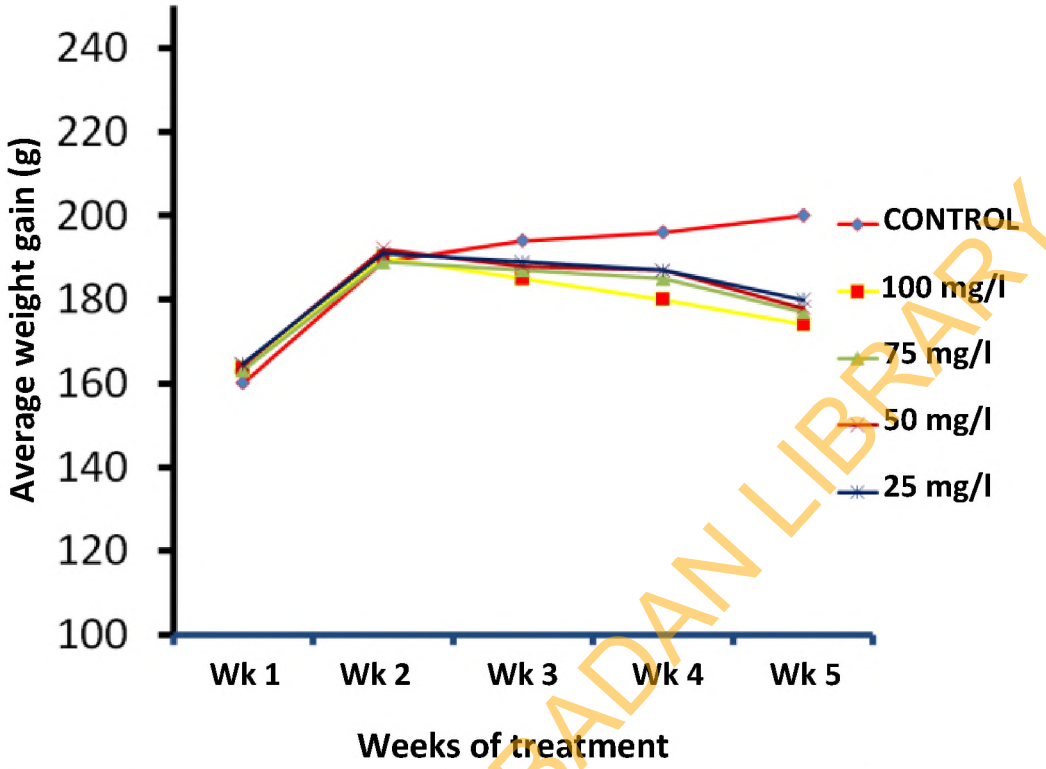

Figure 1. Effect of varying concentrations of LTC on average weight of male *Rattus norvegicus*.

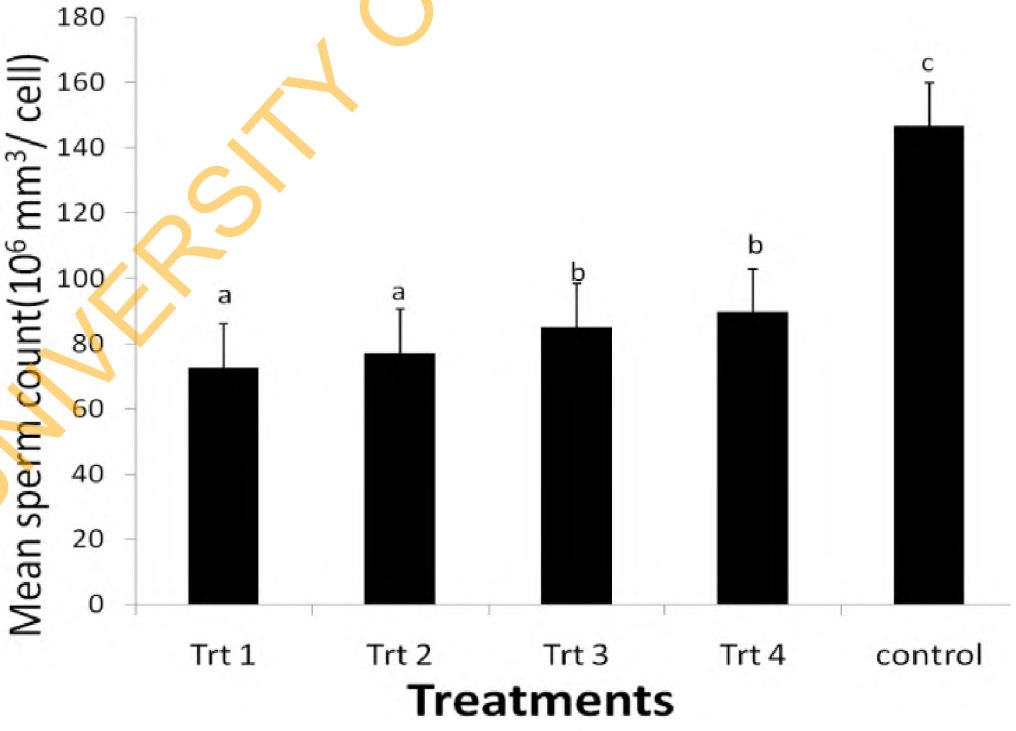

Figure 2. Effect of varying concentrations of LCT on the sperm count of male *Rattus norvegicus*. Bars with the same letter(s) are not significantly different at P<0.05 using DMRT.

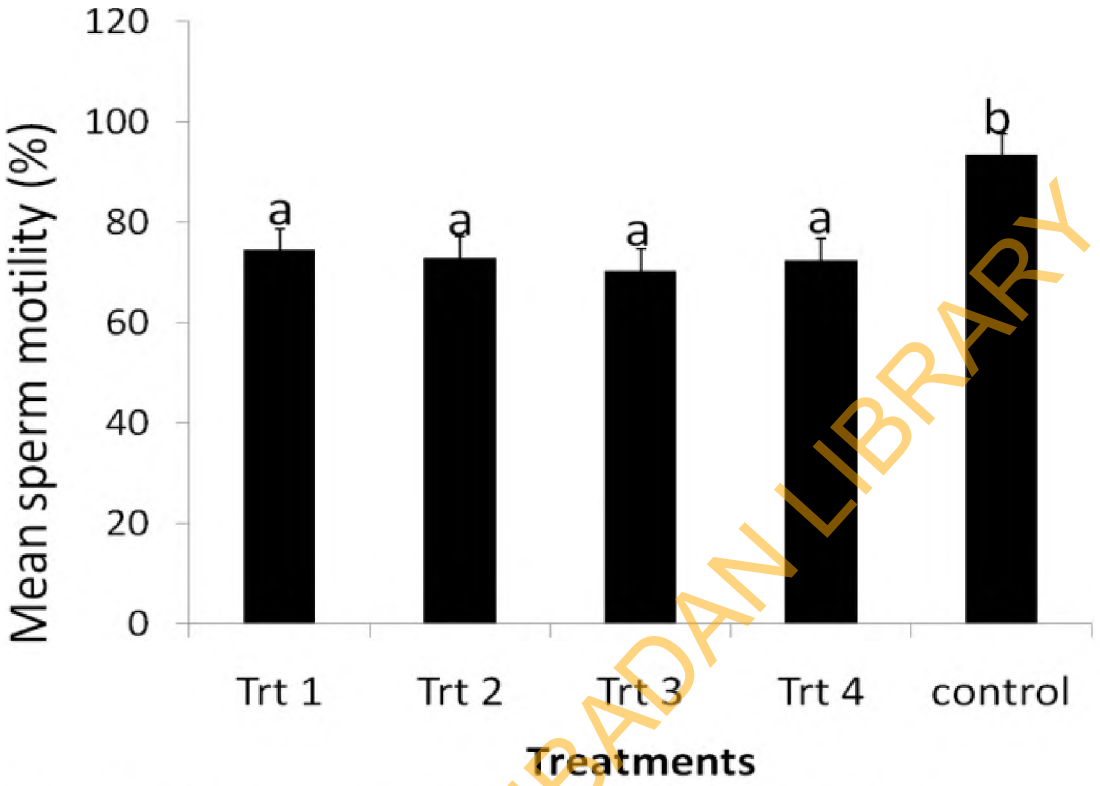

Figure 3. Effect of varying concentrations of LCT on the sperm motility of *Rattus norvegicus*. Bars with the same letter(s) are not significantly different at P<0.05 using DMRT.

Table 1. Toxicity effects of varying concentrations of LTC on spermatogenic parameters of male Wistar rats.

Treatments (mg/kg)	L-D ratio	ASC	Volume
100	77.50±3.31b	15.10±0.36a	5.1±0.13a
75	85.75±3.90ab	13.35±0.37ab	5.1±0.36a
50	87.50±2.22ab	12.78±0.36ab	5.1±0.18a
25	88.75±4.18ab	12.41±0.58ab	5.2±0.29a
Control	97.25±2.64a	10.15±0.12b	5.2±0.27a

L–D, Live–Dead; ASC, Abnormal sperm cell.
Means within the same column followed by the same letter(s) are not significantly different at P<0.05 using DMRT.

Table 2. Effect of varying concentrations of LTC on absolute testes and seminal vesicle weights (g) of Wistar rats.

Treatments (mg/kg)	Seminal vesicle	Left Testes	Right testes
100	1.22 ±0.14a	0.91 ±0.10b	0.91 ±0.09b
75	1.17± 0.14b	1.16± 0.13b	1.15± 0.11ab
50	1.17± 0.35b	1.17± 0.04b	1.15± 0.11ab
25	1.15± 0.23b	1.15± 0.27b	1.16± 0.19ab
Control	1.15 ±0.27b	1.23± 0.14a	1.24 ± 0.23a

Means within the same column followed by the same letter(s) are not significantly different at P<0.05 using DMRT.

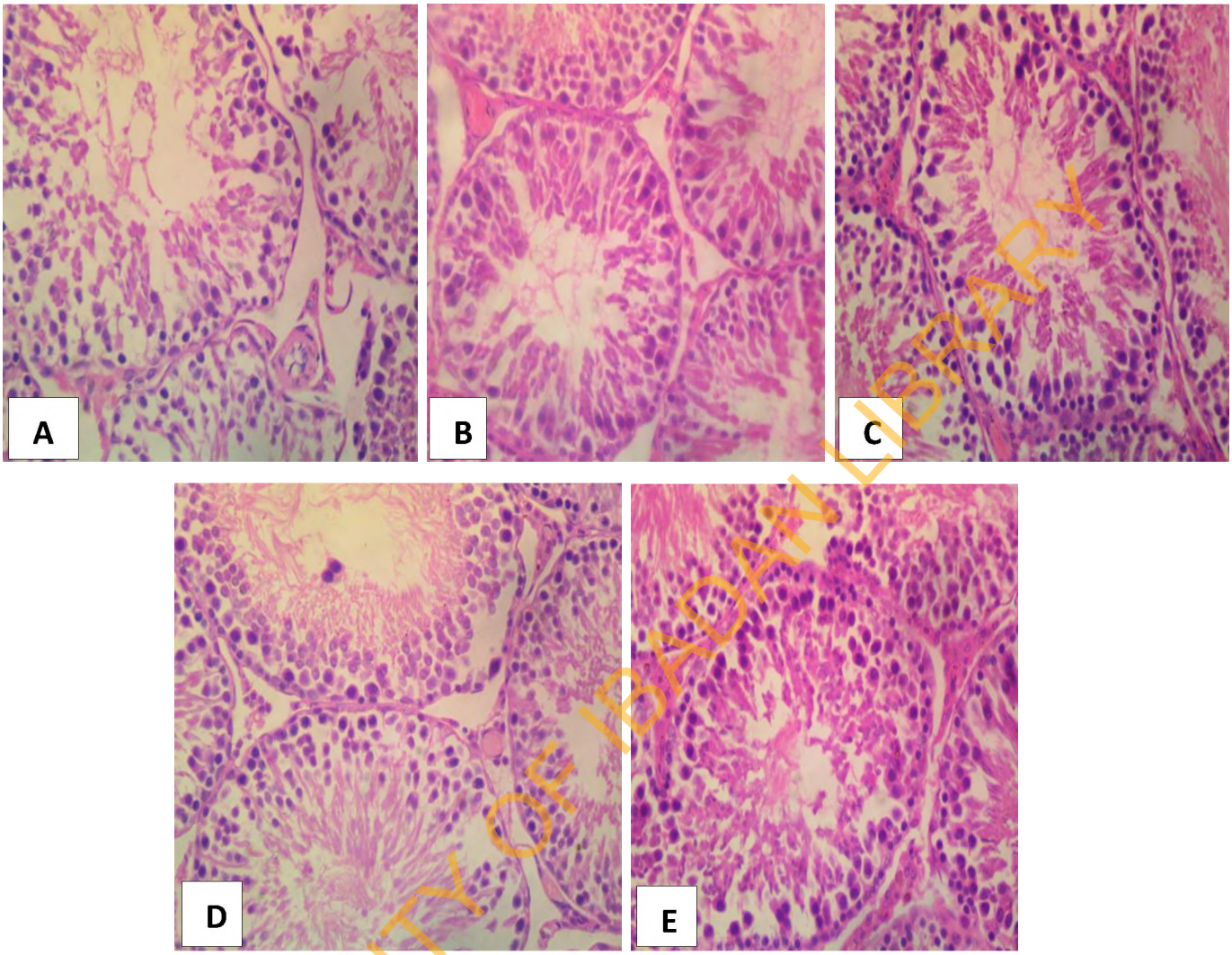

Figure 4. Histological section of testes of *Rattus norvegicus*. A - 100 mg/kg, Variably-sized seminiferous tubules (STs) (containing depleted amounts of spermatogenic cells) with a few having distorted outlines; B - 75 mg/kg, variably-sized STs (containing depleted amounts of spermatogenic cells) with a few having distorted outlines; C - 50 mg/kg, there is mild congestion of interstitial blood vessels; D - 25 mg/kg, there are numerous closely-packed uniformly-sized STs, some of which have irregular outlines. The STs contain slightly depleted amounts of spermatogenic cells; E - Control, numerous closely-packed uniformly-sized STs (packed full with numerous spermatogenic Cells) with regular outlines.

DISCUSSION

The absolute weight of testes and seminal vesicles on all treated animals in this study indicate reduction which may be due to the toxic effects of LTC. These findings were in line with those of Yousef 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), found significant reduction in the index weight of testes of exposed animals following exposure to cypermethrin or other closely related type-II pyrethroids.

This present study also revealed that oral administration of LTC at varying concentrations of 100, 75, 50 and 25 mg/kg for five weeks to male Wistar rats induced deleterious effects on organs of male fertility; this is explicated by the significant decrease in seminal vesicles and testicular index weights. The observed decrease in organ absolute weights of male rats in this study may be attributed to the direct cytotoxic action of the insecticide on these tissues. The observed decrease in organ of male rats could also be ascribed to reduced testosterone synthesis and disruption of normal androgen status (Hossain and Richardson, 2011).

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 (Yu et al., 2014).

Histopathological examination of the testes of LTC-treated rats show marked degeneration of spermatogenic cells associated with interstitial necrosis of spermatogenic cells and congestion with interstitial diffuse edema. These observations conform with previous studies in rat showing sexual dysfunction of male reproductive organs, reduction in testosterone levels after pyrethroid treatment (Yousef et al., 2003; Yousef, 2010; Hossain and Richardson, 2011).

Yu et al. (2014) also found that treatment with deltamethrin inhibited sperm production and increased germ cells apoptosis which caused reduction in the fertility of male rats. The observed histopathological results, in LTC-treated animals, might suggest that this chemical exposure causes deterioration of germinal epithelium and seminal vesicle epithelium. In fact, LTC may influence cell damage due to its plasma membrane lipophilicity (Issam et al., 2009).

The accumulation of LTC in target sites (testes and seminal vesicles) may possibly be responsible for various morphological alterations resulting in reduced sperm count and reduced sperm motility, and hence reduction in spermatogenesis, this is in line with the findings of Prasad et al. (1995). Testicular tissue analyzed by light microscopy in LTC-treated rats show cells that were irregularly spaced and there were also marked intercellular spaces between spermatogenic cells. Although spermatogenesis was present, cell disorganization was largely found. This results of anomalies evident in the experiment thus conformed with findings of Prasad et al. (1995). This would generally result in reduced viability of the sperm, thus resulting in poor fertility. Offspring thus produced are likely to be weak and more prone to disease and immune system disorder.

Conclusion

The five weeks sub-chronic toxicity studies of continuous exposure of rats by oral gavage to different concentrations of LTC suggest dose-related toxic effects on the selected parameters.

The data obtained from the study demonstrate the detrimental effects of LCT on male reproductive organs following repeated exposure to this insecticide. All pesticides must be toxic, or poisonous, to be effective against the pests they are intended to control and due to this, they are potentially hazardous to humans, animals, other organisms and the environment. Therefore, people who use pesticides or regularly come in contact with them must understand the relative toxicity and potential health risk associated with these pesticides.

All directions, restrictions and precautions on pesticide labels should be observed. It is dangerous, wasteful and illegal to do otherwise. The widespread and extensive use of pyrethroid pesticides such as LTC especially in developing nations raises the likelihood of exposure with consequent toxic effect. In most of these countries, the magnitude of pesticide residue in food is largely unknown, hence increasing the risk of exposure.

REFERENCES

Avwioro O. G. (2010). Histochemistry and tissue pathology: Principles and techniques. 1st ed. Nigeria: Claverianum press.

Bancroft J. D. & Gamble M. (2002). Theory and practice of histological techniques. 5pp.

Colombo R., Ferreira T. C. R., Alves S. A., Carneiro R. L. & Lanza M. R. V. (2013). Application of the response surface and desirability design to the Lambda-cyalothrin degradation using photo-Fenton reaction. J. Environ. Manage 118:32-39

Dahamna S., Harzallah D., Guemache A. & Sekfali N. (2009). Toxico-pathological effects in rats induced by Lambda-cyhalothrin. Comm. Agr. Appl. Biol. Sci. 74(1):143-148.

Duncan D. B. (1955). Multiple range and multiple F tests. Biometrics 11:1-42.

El-Demerdash F. M.(2007). Lambda-cyalothrininduced changes in oxcidative stress biomarkers in rabbit erythrocytes and allevation effect of some antioxidants. Toxicol. In-Vitro 21:392-397.

Fadina O. O., Taiwo V. O. & Ogunsanmi A. O. (1999). Single and repetitive oral administration of common pesticides and alcohol on rabbits. Trop. Vet. 17:97-106.

Feustan M. H., Bodnai K. R. & Kerstetter S. L. (1989). Reproductive toxicity of 2-methoxy ethanol applied dermally to occluded and non-occluded sides in male rats. Toxicol. Appl. 23:45-65.

Gupte S. M., Borah D. K. & Motto H. L. (1991). Preferential flow and contaminant migration in a new Jersey Field. Proceedings of the National Symposium, December 16-17, Chicago, Illinois. Pp.173-182.

Hayat M. (1989). Principles and techniques of electron microscopy, biological applications. 3rd ed. CRC press. 24-74.

Hossain M. M. & Richardson J. R. (2011). Mechanism of pyrethroid pesticide-induced apoptosis: role of calpain and the ER stress pathway. Toxicol. Sci. 122:512-525.

Issam C., Samir H., Zohra H., Monia Z. & Hassen B. C. (2009). Toxic responses to deltamethrin (DM) low doses on gonads, sex hormones and lipoperoxidation in male rats following subcutaneous treatments. J. Toxicol. Sci. 34:663-670.

Kilian Delport E. R., Bornman M. S. & De Jager C. (2007). Simultaneous exposure to low concentrations of dichlorodiphenyl trichloroe - thane, deltamethrin, nonylphenol and phytoestrogens has negative effects on the reproductive parameters in male Sprague-Dawley rats. Andrologia. 39:128-35.

Krzanowska H., Styrna J. & Wabik-Silz B. (1995). Analysis of sperm quality in recombinant in bred mouse stain: Correlation of sperm head shape with sperm abnormalities and with incidence of supplementary spermatozoa in the perivitelline space. J. Reprod. Fertil. 104:347-354.

Lee N., McAdam D. P. & Skeritt J. H. (1998). Development of Immunoassays for Type II synthetic pyrethroids 1 and 2. J. Agr. Food Chem. 46:520-546.

Linder R. E., Strader L. F. & McElroy W. K. (1986). Measurement of epididymal sperm motility as a test variable in the rat. Bull. Environ. Contam. Toxicol. 38(3):317-324.

Oros D. R. & Werner I. (2005). Pyrethroid insecticide: An analysis of

use pattern, distribution, potential toxicity and fate in the Sacramento. San Joaquin Delta and Central Valley. White paper for the Interagency Ecological Program. SFEI contribution 415. San Francisco Estuary Institute, Oakland, C.A.

Petrie A. & Watson P. (1999). Statistics for veterinary and animal science. 1st ed., pp 9099 The Blackwell Science Ltd., UK.

Prasad A. K., Pant N., Srivastava S. C., Kumar R. & Srivastava S. P. (1995). Effect of oral administration of carbofuran on male reproductive system of rat. Hum. Exp. Tol. 14:484-491.

Southwood J. (1985). Acute oral toxicity studies. Zeneca agricultural products laboratory project no. CTL/P/1102. 321p.

Thatheyus A. J. & Selvam A. D. G. (2013). Synthetic pyrethroids: Toxicity and biodegradation. Appl. Ecol. Environ. Sci. 1(3):33-36.

Yousef I. Y. (2010). Vitamin E. Modulates reproductive toxicity of pyrethroid Lambda-cyhalothrin in male rabbits. Food Chem. Toxicol. 48:1152-1159.

Yousef M. I., El-Demerdash F. M., Kamel K. I. & Al-Salhen K. S. (2003). Changes in some hematological and biochemical indices of rabbits induced by isoflavones and cypermethrin. Toxicology 189:223-234.

Yu H. M., Wu Y., Ju P., Wang B. H., Yang X. D., Wang H. M. & Xu L. C. (2014). eNOS-JNK1-AR signaling pathway mediates deltamethrin-induced germ cells apoptosis in testes of adult rats. Environ. Toxicol. Pharmacol. 38:733-741.