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Article

The Potential Negative Effect of a Lambda-Cyhalothrin Pesticide on Some Testicular Functions in Male Albino Rats

Duha Zeki Al-Swefee*[1]
Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq
*Correspondence: doha.abd@sc.uobaghdad.edu.iq

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Abstract: In this study, the triggered toxicity of the pyrethroid pesticide lambda-cyhalothrin (LCT) was investigated in male albino rats. The total number of adult's rat applied in the experiment was Fifteen, separated into three groups and orally administered LCT at doses 0, 15, and 30 mg/kg for 30 days. Serum reproductive hormones, oxidative stress biomarkers, and sperm functional parameters were evaluated during the laboratory experiment. LCT exposure resulted in a significant elevation of prolactin levels, while estrogen remained unchanged. Two doses of LCT were applied, the result shows a highly significant reduction in FSH, LH, and testosterone concentrations, indicating disruption of the hypothalamic-pituitary-gonadal axis. Oxidative stress was markedly increased, as evidenced by elevated levels of malondialdehyde (MDA), catalase (CAT), glutathione (GSH). The laboratory tests of sperms indicated decline in total sperm count and motility, as well by increased percentages of dead and morphologically abnormal sperm in treated groups. These findings demonstrate that LCT induces pronounced endocrine disruption, oxidative imbalance, and deterioration of sperm quality, confirming its strong reproductive toxicity in male rats. The study emphasizes the potential risk of LCT exposure to male fertility and highlights the need for stricter regulation and reduced environmental use of pyrethroid pesticides.

Keywords Lambda-Cyahlothrin, Reproductive Toxicity, Male Albino Rat, Testosterone

Introduction

The major challenges in the world using sustainable particles is the increased rate of food production to cover the hunger of the increasing population [1], [2]. Pesticides are chemicals due to their widespread and accumulation along the food chain represent significant risks to mammals as non-target organisms [3]. Male infertility constitutes a significant and persistent global issue. Several investigations claimed that the exposure of humans to a various of environmental pollutants, including pyrethroids, induces multiple male reproductive problems [4]. Pyrethroids are used to protect the crops against insects. Lambda-cyhalothrin (LCT) is an artificial pyrethroid of type II pesticide utilized for the efficient elimination of insects in homes and agricultural settings [5], [6]. Pyrethroids have constantly replaced organophosphorus pesticides and now constitute the most prevalent insecticides globally [7]. Pyrethroids are classified as endocrine-disrupting chemicals (EDCs) and have been implicated in impairing male reproductive function [8]. The World Health Organisation recommendation was LCT as a moderately

toxic (class ii) technical-graded active ingredient in insecticides [9]. LCT product guidelines suggest that it poses reduced risk to non-target organisms; however, LCT is not devoid of adverse effects [10]. It induces significant changes in hematological parameters and elevates oxidative stress biomarkers, including lactate dehydrogenase (LDH), malondialdehyde (MDA), and glutathione peroxidase (GSH), in rats exposed to of LCT [11], [12]. Exposure to pyrethroids, such as LCT in rats, and cypermethrin in Swiss albino mice has been shown testicular toxicity and structural alterations in spermatozoa [13], 14]. Furthermore, occupational exposure to pesticides has been linked to reduced semen quality and infertility among greenhouse workers [15].

Recent studies have increasingly examined the reproductive and endocrine risks associated with pyrethroids, particularly following their classification as direct or indirect endocrine disruptors. Various pyrethroids and their metabolites have the potential to disrupt hormone receptors, thereby interfering with the endocrine reproductive system [16], 17]. A recent study demonstrated that occupational exposure to pyrethroids adversely affects semen quality in workers [18]. Numerous investigations in mammalian models further support these findings. For example, exposure to cypermethrin has been associated with reductions in key endocrine hormones-including testosterone, luteinizing hormone(LH), and follicle-stimulating hormone (FSH)- as well as decreased weights of the testes and epididymis in rats [19]. Kilian et al. examined the effects of deltamethrin and phytoestrogens on reproductive parameters in rats and reported that both compounds altered sperm count and testicular mass. The authors further suggested that deltamethrin may exert estrogenic like activity [20]. However, considerable evidence indicates that humans face a heightened risk of exposure to LCT [21]. Experimental studies Research indicates that exposure of male mice to LCT adversely affects testicular architecture, as indicated by Leydig cell degeneration and alterations in sperm morphology, quality, and quantity [22]. Yousef reported that exposure of male rabbits to LCT adversely impacts reproductive organ weights, reduces serum testosterone levels, and is associate with increased testicular oxidative stress [23].

Materials and Methods

Experimental design

The research was performed to determine the adverse impact of LCT pesticide on some physiological parameters and testicular functions in male rats. This study included 15 albino male rats, weighing 180-190g and 5-6 months' age, divided into three groups as follows:

The first group was considered a control group and was given only water and food. The 2nd and 3rd groups were given LCT pesticide at concentrations of 15 and 30 mg/kg, respectively. Treated animals were dozed orally for 30 days. The experiments were terminated after 30 days. At the end of the experiment, blood was drawn from the heart after anesthetizing the animals with ketamine at a concentration of 10%. The blood was separated through centrifugation for 10 minutes at a speed of 3000 rpm. The resultant serum was preserved in the refrigerator at -20 degrees until the biochemical analyzes were performed, which included Estrogen, Prolactin, MDA, CAT, GSH, FSH, LH, and Testosterone, were performed. Some criteria were measured for the sperm, while others included the total count, viability, motility and abnormality.

Biochemical testes

1. 1-Malondialdyhide My BioSource/USA
2. 2-Catalase Human Catalase (CAT) ELISA Kit My BioSource/USA

Hormone analyses

LCT pesticide Elisa kit (My BioSource/USA) was used to estimate serum testosterone hormone concentration. Elisa kits provide by(Human-Germany) was used to measure serum follicle stimulating hormone and serum luteinizing hormone concentrations.

Epididymal tail suspension preparation

To prepare the suspension, the epididymis was separated, placed in theRPMI-1640 medium at37 c°, and cut into very small pieces by micro-surgical scissors to allow the sperm to exit into the RPMI -1640medium [24]. The following tests were performed on it:

Sperm function

A. Sperm count

The total sperm count was calculated by counting the number of sperm in five squares directly using an optical microscope at high magnification, then calculating the average number of sperm in five fields and multiplying the result X $10^{6.}$

Total count of sperm= Average sperm count X $10^{6.}$ (direct method) [24].

B. Sperm motility

To determine sperm motility and counts, 100 mg of caudal epididymis was minced in 1 ml of RPMI-1640. One drop of mixed sample was applied on slide under a cover slip, the sperm motility was investigated by counting both motile and immotile spermatozoa per unit area and expressed as an index.

C. Examining the morphology of dead and abnormal sperms

A morphological test was performed to assess dead, abnormal and living sperms. one drop of sperm suspension previously prepared, was placed over the edge of slide and mixed with an equal volume of easion-nigrosin stain. Then, two smears of each sample were prepared on microscopic slide. The sperm smears were examined for abnormal morphology under the microscope, 200 sperm were counted in each smear. The final percentage was estimated through calculating the average of two smears [25]. Abnormal sperm morphology and alive and dead sperms was calculated according to the following equations.

Percentage of dead sperm= No. of dead sperm /Total sperm no. * 100

Percentage of abnormal sperm= No. of morphologically abnormal sperm /Total sperm no. * 100

Statistical Analysis

The Statistical Packages of Social Sciences-SPSS (2019) program was used to detect the effect of difference concentration/ Treatments in study parameters. LSD-Least significant difference and Duncan's test was used to significant compare between means in this study [26].

Results

Table 1. Comparison between difference concentrations of LCT pesticide on Estrogen and Prolactin

Conc. (mg/kg)	Means ±SE	
	Estrogen (pg/ml)	Prolactin (ng/ml)
0 mg/kg	194.03 ±10.29	24.37± 0.88 b
15 mg/kg	183.00 ±2.89	23.87 ±3.24 b
30 mg/kg	183.67 ±4.17	34.10 ±2.51 a
L.S.D.	22.933 NS	8.372 *
P-value	0.4651	0.0413

Different letters indicate comparison in same column. * (P≤0.05).

Statistical analyses showed that there's significant difference in prolactin level of treated group with LCT pesticide in 15 and 30 mg/kg (23.87 ±3.24, 34.10 ±2.51) ng/ml respectively, compared with control group (24.37± 0.88) ng/ml, while the statistical analyses of Estrogen were non-significant (Table 1).

Table 2. Comparison between difference concentration of LCT pesticide on sex hormones (FSH, LH and Testosterone) in male rats

Conc. (mg/kg)	Means ±SE		
	FSH (mIU/ml)	LH (mIU/ml)	Testosterone (ng/ml)
0 mg/kg	44.20 ±2.65 a	4.52 ±0.27 a	6.82 ±0.40 a

15 mg/kg	28.78 ±1.71 b	3.12 ±0.50 b	2.57 ±0.53 b
30 mg/kg	22.28 ±1.44 b	2.28 ±0.23 b	2.73 ±0.34 b
L.S.D.	6.948 **	1.231 **	1.502 **
P-value	0.0007	0.0101	0.0008

Different letters indicate comparison in same column. ** (P≤0.01).

A highly significant decrease was noticed in FSH, LH and Testosterone hormones of the treated groups with LCT pesticide in 15 and 30 mg/kg (28.78 ±1.71, 22.28 ±1.44), (3.12 ±0.50, 2.28 ±0.23) mIU/ml and (2.57 ±0.53, 2.73 ±0.34) ng/ml respectively compared to the control group (44.20 ±2.65), (4.52 ±0.27) mIU/ml and (6.82 ±0.40) ng/ml respectively (Table 2).

Table 3. Effect of different concentrations of LCT pesticide on antioxidant enzymes in male rats

Conc. (mg/kg)	Means ±SE		
	MDA (uml\L)	CAT (IU/L)	GSH (uml\L)
0 mg/kg	1.756 ±0.17 c	3.43 ±0.35 c	65.58 ±2.57 b
15 mg/kg	4.86 ±0.33 b	9.66 ±0.86 b	79.99 ±4.90 a
30 mg/kg	6.77 ±0.46 a	14.24 ±1.23 a	83.57 ±3.04 a
L.S.D.	1.190 **	3.095 **	12.632 *
P-value	0.0001	0.0004	0.0286

Different letters indicate comparison in same column. * (P≤0.05), ** (P≤0.01).

Statistical data also revealed a highly significant increase in the MDA and CAT level of LCT pesticide treated groups scoring 15 and 30 mg/kg (4.86 ±0.33, 6.77 ±0.46) uml\L, (9.66 ±0.86, 14.24 ±1.23) IU/L respectively with control group (1.756 ±0.17) uml\L and (3.43 ±0.35) IU/L. Also a significant increase in GSH level of treated groups compared to control group (Table 3).

Table 4. The effect of different concentrations of LCT pesticide on sperm characteristics in male rats

Conc. (mg/kg)	Means ±SE			
	Total count $x10^6$	Motility (%)	Dead (%)	Abnormality (%)
0 mg/kg	36.33 ±1.85 a	93.33 ±1.66 a	6.67 ±0.88 b	5.33 ±0.88 b
15 mg/kg	28.33 ±2.40 b	80.00 ±2.89 b	18.00 ±3.00 a	16.00 ±1.15 a
30 mg/kg	26.33 ±1.85 b	71.67 ±3.33 b	16.67 ±1.76 a	20.33 ±2.02 a
L.S.D.	7.110 *	9.418 **	7.172 **	4.983 **
P-value	0.0302	0.0039	0.0101	0.0008

Different letters indicate comparison in same column. * (P≤0.05), ** (P≤0.01).

The results showed a significant decrease in the total number of sperm treated with the concentrations of insecticide 15 and 30 mg/kg (28.33 ±2.40, 26.33 ±1.85) sperm $x10^6$ respectively compare to control group (36.33 ±1.85) sperm $x10^6$ the results also showed a highly significant decrease in motility percent and increase in the percentage of dead and abnormal sperm (Table 4).

Discussion

Recent investigations demonstrate that pyrethroid insecticides exert significant adverse effects on male reproductive health. These compounds have been associated with reduction in sperm count and motility, as well as structural deformities in sperm head morphology. They also increase the proportion of morphologically abnormal sperm and induce DNA damage, including higher rates of sperm aneuploidy. Furthermore, pyrethroid can alter circulating sex hormone levels, collectively contributing to marked reproductive toxicity [27].

The current study demonstrates that the exposure to LCT induces marked effects in reproductive hormones, increases oxidative stress biomarkers, and negatively affects sperm functional parameters in male rats, that's agree with previous study on pyrethroid insecticides. A significant reduction in serum FSH, LH and testosterone levels in rats treated with both doses of LCT. This hormonal suppression supports the classification of pyrthroid as endocrine disrupting chemicals that interfere with the hypothalamic pituitary gonadal axis. Yousef reported a marked decline in testosterone in male rabbits exposed to LCT due to degeneration of leydig cells [23]. High exposure to cypermethrin lead to reduce in LH, FSH, and testosterone in rats [14]. The levels of testosterone were decline, observed in the present study may result from direct testicular damage, affecting Leydig cell steroidogenesis and central inhibition of gonadotropins, as indicated by the reduction in LH and FSH. prolactin is known to suppress hypothalamic release of GnRH, which subsequently reduces LH and FSH secretion. Kumar *et al*., who reported increased prolactin following pyrethroid exposure [14]. In contrast, estrogen levels did not show significant alterations, which is expected since male rats produce minimal estrogen and LCT primarily targets androgenic rather than estrogenic pathways. The results also demonstrated a significant increase in MDA, accompanied by elevated CAT and GSH levels in LCT treated rats. Increased MDA indicates enhanced lipid peroxidation, reflecting oxidative injury to testicular cellular membranes. The elevation in CAT and GSH likely represents a compensatory antioxidant response triggered by excessive production of reactive oxygen species (ROS). Pyrethroids are well known to generate ROS, leading to cellular damage, DNA fragmentation, and impaired spermatogenesis [3]. A significant decline in total sperm count and motility was observed, along with an increase in abnormal and dead sperm. These parameters are highly sensitive indicators of testicular toxicity that may contributed to oxidative damage to germ cells and sperm membranes due to increased ROS and mitochondrial dysfunction in sperm midpiece, reducing motility. Ratnasooriya *et al*. found decreased sperm quality and increased morphological abnormalities after cyhalothrin exposure [13]. Boumezrag *et al* confirmed that LCT disrupts sperm viability, morphology, and count [6].

Conclusion

In this study, we indicate that the pesticide lambda-cyhalothrin exerts potent, multi-level reproductive toxicity in male albino rats. LCT disrupted the hypothalamic pituitary gonadal axis, induced pronounced oxidative stress, and severely compromised spermatogenesis, as reflected by suppressed gonadotropins, reduced the levels of testosterone, elevated lipid peroxidation, and marked sever damage in sperm quality. These converging endocrine and oxidative disturbances confirm LCT as a robust reproductive toxicant. Given the substantial utilization of pyrethroids, the present outcomes demonstrate an urgent need to reassess exposure risks and further investigate the mechanistic and transitional implications for human male fertility.

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