Genotoxic effects of Lambada-cyhalothrin& Dimethoateon adult Male RatsFertility

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Abstract

Background: Continuous use of pesticides could have adverse effects on the reproductive functions of humans and non-target organisms.**Objective:** This study was carried out to examine the effect of Lambada-cyhalothrin & Dimethoateon the reproductive parameters of adult male albino rats. **Methods:** Forty (40) adult male Wistar albino rats weighing between 180 – 200g were divided into 5 groups of 8 animals each. Two groups were given Lambada-cyhalothrin (20, 40mg/kg) respectively, and Two groups were given Dimethoate (20, 40mg/kg) respectively. The control group was given drinking water. All treated daily by oral gavage for 30 days. **Results:** The results indicated the presence of a significant decrease ($P<0.05$) in the number of sperms and a significant increase in the number of abnormal spermatozoa in rats exposed to Lambada-cyhalothrin & Dimethoatewhen compared with control group, treated with drinking water.There were significant reductions ($p<0.05$) inon sex hormones level which includingfollicle-stimulating hormone (FSH), luteinizing hormone(LH) and testosterone levels in male rats exposed to Lambada-cyhalothrin & Dimethoatewhen compared with control group, which treated with drinking water.The testicularhistology of animals treated with Lambada-cyhalothrin & Dimethoategenerally showedtubular atrophy with loss of spermatogenesis, the effect grading as mild in cases loss of spermatogenesis. The results confirmed that dimethoate seriously deteriorate the malereproductive system resulting in decreased fertility.

Conclusion: Tested pesticides may result in anomalies of the male reproductive system, such as decreased sperm production. Prior to fertilization, it's also conceivable that the sperm's genetic makeup could be changed.

Keywords: Lambada-cyhalothrin, Dimethoate, fertility, testosterone, FSH, LH, Genotoxicity, albino rats.

1. Introduction

Infertility in men is a serious, ongoing problem all across the world. Studies have shown that exposure to pyrethroids and other environmental contaminants causes a range of problems in male reproduction[(1)].Pyrethroids are used to protect crops against pests. Type II pyrethroids, which incorporate cyhalothrin rings, are among the broad-spectrum, very effective organic insecticides that are widely used in domestic, veterinary, and agricultural applications. Lambda-cyhalothrin (LCT), a synthetic type II pyrethroid insecticide, is frequently used to eradicate pests in both food and non-food crops as well as disease-carrying insects, ticks, and flies. There is convincing evidence to suggest that LCT exposure is now more dangerous for people[(2)].

Infertility in men is a serious, ongoing problem all across the world. Studies have shown that exposure to pyrethroids and other environmental contaminants causes a range of problems in male reproduction[(3)]. Leydig cell degeneration and alterations in the quantity, quality, and appearance of sperm produced by male mice exposed to LCT have been used to establish that this substance has a detrimental impact on the testicular architecture and has a negative impact on reproduction[(4)]. Male rabbits exposed to LCT exhibited lower serum testosterone levels and more oxidative stress in their testicles, which affected the weights of their reproductive organs, according to studies by Yousef[(5)].These results suggest that the male reproductive toxicity of LCT, which results in reduced testosterone levels and the generation of oxidative stress, is at least partially responsible.

The Food and Agriculture Organization (FAO) defines a pesticide as "Any substance or mixture of substances intended for preventing, killing, or Because of its fast

biodegradation and reduced environmental persistence, organophosphate insecticides have replaced organochlorine pesticides. Some of the most widely used insecticides in the world are manufacturedof organophosphorus and are easily available commercially for both home and industrial usage. Half of the insecticides used globally are applied there. However, due to their extensive usage in agriculture and public health, these pesticides eventually find their way into the ecosystem and have an effect on the local animals. Phosphates, phorodiphosphates, and phosphorothioates are the three different forms of solid and liquid organophosphorus compounds[6]. The possibility that dimethoate may have an effect on people and wildlife in their natural surroundings is therefore quite concerning. It has been demonstrated that dimethoate can harm developing organisms and reduce their capacity to reproduce when exposed repeatedly. Dimethoate has developmental toxicity that affects fetal weights, resorption rates, implantation rates, and live birth rates[7].In both sexes of adult rats, this pesticide has been demonstrated to be harmful to reproduction. In males, reports of decreased fertility, suppressed libido, declining semen quality, altered testosterone levels, and testicular degeneration have been made[8].In females, irregularities in the Estrous cycle and altered serum gonadotrophin levels have also been noted. Genotoxicity is the capacity of a substance to alter genetic elements at non-toxic amounts with the potential that this alteration will occur during cell division (9). Multiple genetic tests are recommended by standard methodology since it is insufficient to assess the toxicity of a substance using just one parameter. One test that demonstrates DNA damage after chemical exposure is the comet assay. Because of its significance for biomonitoring, it is appropriate, quick, and extensively used (10).

This study evaluated the genotoxicity of testicular tissue in adult male albino rats and the reproductive toxicity of lambada-cyhalothrin and dimethoate

2. Materials and methods

2.1. Experimental protocol

The animals were divided into five equal groups, each group consist of 8 rats. All treated daily by oral gavage for 30 days as follow :

Group1: (control group) was given drinking water.

Group2(L1)& Group3(L2): were given (20,40mg/kg) respectively, of Lambada-cyhalothrin. The LD50 in our study was 612 mg/kg body weight, which has been used previously by other authors[11].

Group4(D1)& Group5(D2): were given Dimethoate (20, 40mg/kg) respectively, of Dimethoate. The LD50 in our study was 200 mg/kg body weight, which has been used previously by other authors[12].

2.2. Blood collection:

After thirty days of treatment, the animals were sacrificed. subsequently, the blood samples were collected by cardiac puncture, 5mL of blood were drawn from each animal of experimental groups, and put in tubes without EDTA, centrifugedat 3000 rpm for 15 minutes, and then serum was separated and kept in the refrigerator at -20ºC until the time of assay.

2.3. Numbers and abnormality of sperms:

Fourty male wistar rats weighting (180 – 200) grams were used in this experimental study. To determination the effect of Lambada-cyhalothrin& dimethoatein sperm concentration Soto' s method[13]was used , and Wyrobek and Bruce' s method[14] used to determination sperm abnormalities.

2.4. Hormone assay:

Serum samples were analyzed for FSH and LH concentrations, through solid phase ELISA based on the principle of competitive binding, using commercial kits from VEDALAB (France), while for measurement of testosterone using kit from Bio Meriux(France).

2.5. Determination of DNA damage by comet assay

The alkaline comet assay was carried out as described by Olive and Banath[15] and Speit and Rothfuss[16]with some modifications. Immediately after euthanasia, 1 g of testis was placed in a Petri dish, add to it 1 ml of cooled cutting solution (NaCl 0.43 gram, EDTA Na 0.8 gram for 100ml distilled waterPH 7.5) and cut it well with scissors. Transfer the whole mixture to the Abendrov tube and mix gently with the homogenizer (the tube is placed on ice) 500 rpm Label slide on frosted end using a pencil, not a pen.Pipet 0.1 ml of cells into a 5 ml plastic disposable tube.Add 1.2 ml 1% low-gelling-temperature agarose at 40 °C Mix and rapidly pipet 1.2 ml of cell suspension onto the agarose-covered surface of a pre-coated slide; avoid producing bubbles. The agarose was allowed to set at 40°C for 2 min and the slides were immersed in lysis solution (1.2 M NaCl, 100 mM EDTA, NaOH to pH >13) with freshly added 1% Triton X-100 and 1%

DMSO at 4°C overnight. Slides were then placed side by side on the horizontal gel box along with filling the buffer reservoirs with freshly made pH>12.3 electrophoresis buffer containing 0.03 M NaOH and 2 Mm Na EDTA for 25 min before electrophoresis at 0.6 V/cm. Remove slides from electrophoresis chamber and rinse and neutralize in 400 ml of distilled water. Place slides in staining solution containing 2.5 µg/ml of red safe in distilled water for 20 min. Rinse slides with 400 ml distilled water to remove excess stain. The slides were examined by fluorescent microscope.

2.6 Histological Study :

After the collection of blood samples from the animals the following organ liver was isolated In brief the routine sequence of events according to Suvarna*et al.,* [17].

Ethical approval: The project was approved by The Local Ethics Committee at Thi Qar University in accordance with University Order No. (5644) on 05/20/2022.

Statistical analysis:

Statistical analyses were done utilizing the computer data processing (SPSS, version 26). A probability value (P<0.05) was considered to be statistically significant. And used to calculate least significant difference (L.S.D.) values for the comparison of means following. According to comet type , the scored comet categorized for five groups depended on the tail DNA percent as describedby Srivastava and Singh, [18]. comet percent and represented histogram were conducted by Microsoft excel 2010 (Microsoft corporation, USA) . the significant difference among different treatment groups were assessed by Chi squire test under P value 0.05 by employing IBM SPSS software v.21 (IBM,USA).

Table 1 : Effect of of Lambada-cyhalothrin& dimethoateon Sperm Properties of male rats.

Treatments mg/kg	Mean ± SD of Sperm Properties	
	Sperm Abnormality %	Sperm Count
Group1(control)	0.140 ± 0.015^{e}	$103.0 *10^{4}\pm 7.6*10^{4a}$
Group2	0.338 ± 0.032^{d}	$77.5 *10^{4}\pm 3.0*10^{4b}$
Group3	0.381 ± 0.024^{c}	$80.6 *10^{4} \pm4.4*10^{4b}$
Group4	0.461 ± 0.019^{b}	$51.8 *10^{4} \pm2.9*10^{4c}$
Group5	0.503 ± 0.036^{a}	$52.1 *10^{4}\pm 3.0*10^{4c}$
p. value	**0.000**	**0.000**
LSD	**0.037**	**54415.8**

3.Results

3.1. Effect of of Lambada-cyhalothrin& dimethoateon Sperm Properties of male rats:

The results indicated the presence of a significant decrease (P<0.05) in the number of sperms and a significant increase (P<0.05) in the number of abnormal spermatozoa in the of the male rats treated with Lambada-cyhalothrin & dimethoate, when compared with control groups. while the rats treated with Dimethoate (20,40) mg/kg showed a significant decrease (P<0.05) in the number of sperms and a significant increase (P<0.05) in the number of abnormal spermatozoa when compared with Lambada-cyhalothrin (20,40) mg/kg (table 1).

Values are means ± S.E.

Different letters refer to significant differences (p<0.05).

Same letters refer to No significant differences (p<0.05).

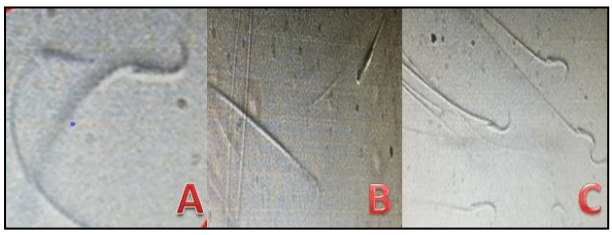


Fig (1)Photomicrograph abnormality of sperms of the male rats treated withLambada-cyhalothrin& dimethoate.Rat spermatozoa: (A) Curved tail, (B) Banana heade and (C) Normal sperm

3.2. Effect of Lambada-cyhalothrin& dimethoateon sex hormone levels of male rats.

The obtained results revealed a significant decrease (p<0.05) in FSH, LH and testosterone hormone levelof the male rats treated withLambada-cyhalothrin&

dimethoate, when compared with control groups (table 2).

Table 2 : Effect of Lambada-cyhalothrin& dimethoateon sex hormone levels of male rats.

Treatments mg/kg	Mean ± SD of Sex Hormones		
	FSH)mlU/ml(	LH)mlU/ml(	Testosterone (ng/ml)
Group1(control)	5.45±0.35[a]	4.46±0.19[a]	22.3± 1.70[a]
Group2	4.15± 0.03[b]	3.62± 0.15[b]	19.5± 1.84[ab]
Group3	4.47± 1.10[b]	3.44± 0.13[bc]	19.7± 3.10[ab]
Group4	3.53± 0.55[c]	3.15± 0.57[c]	18.6± 1.32[b]
Group5	3.50±0.27[c]	2.76±0.46[c]	14.8± 3.56[c]
p. value	**0.000**	**0.00**	**0.000**
LSD	**0.38**	**0.42**	**2.93**

Values are means ± S.E.

Different letters refer to significant differences ($p<0.05$).

Same letters refer to No significant differences ($p<0.05$).

3.3. Effect of dimethoate & Lambada-cyhalothrin on DNA damage of testis of of adult male albino rats.

Classification of comets based on tail DNA%. Categories of comet: (**A**) undamaged cells (tail DNA% <5); (**B**) low damaged cells (tail DNA% 5–25); (**C**) moderately damaged cells (tail DNA% 26–45); (**D**) highly damaged cells (tail DNA% 46–80); (**E**) extremely damaged cells (tail DNA% >80).

Table (3) number and percent of comet types in different treatment groups

		undamaged nomber		low damaged cells		moderately damaged cells		highly damaged cells		extremely damaged cells	
group	total scored cells number	number	%	number	%	number	%	number	%	number	%
Group1 (control)	247	227	91.90 %	18	7.29 %	2	0.81 %	0	0.00 %	0	0.00 %
Group2	269	11	4.09 %	48	17.84%	45	16.73%	86	31.97%	79	29.37%
Group3	332	22	6.63 %	57	17.17%	61	18.37%	103	31.02%	89	26.81%
Group4	239	8	3.35 %	33	13.81%	51	21.34%	78	32.64%	69	28.87%
Group5	322	6	1.86 %	42	13.04%	63	19.57%	124	38.51%	87	27.02%

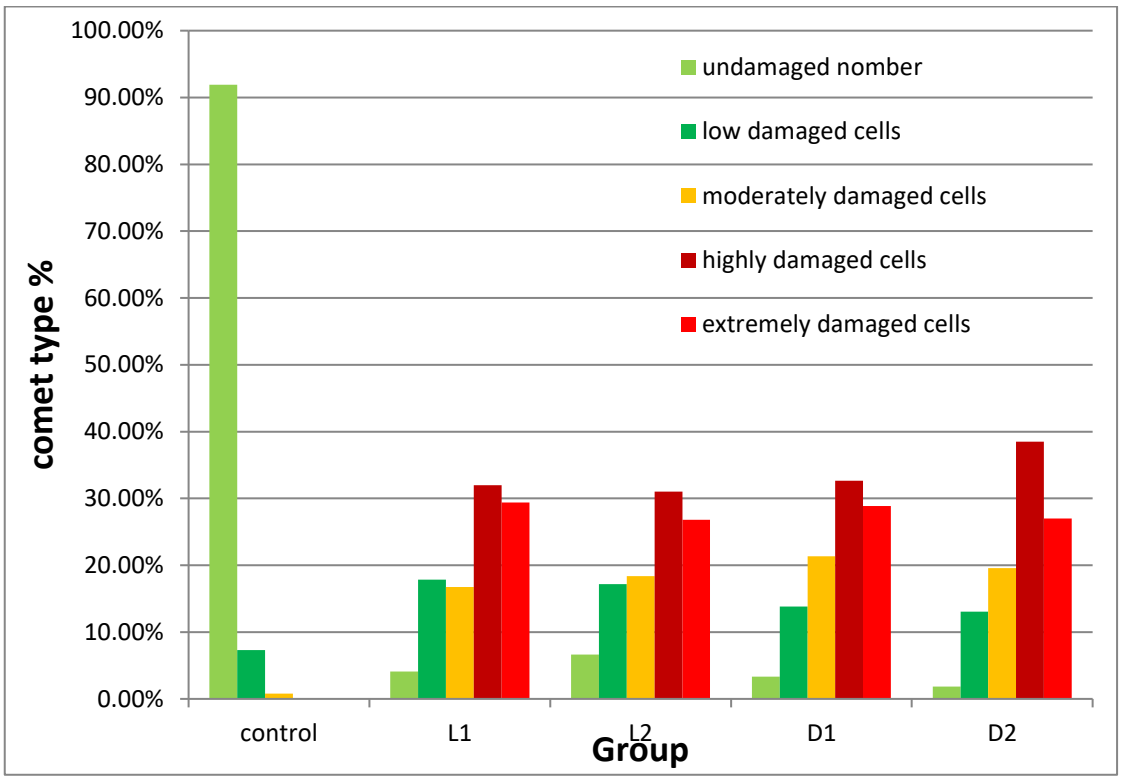
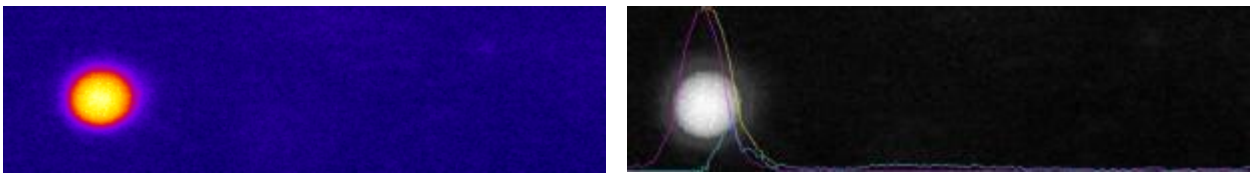


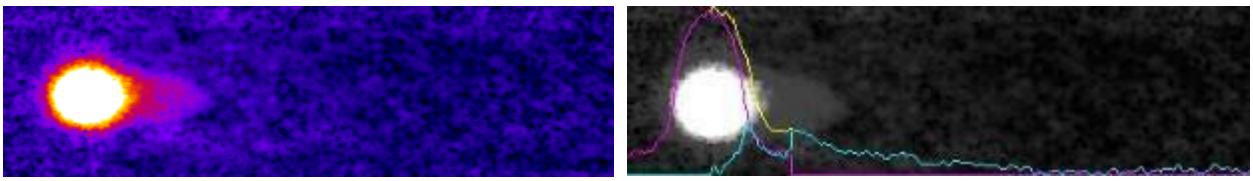
Figure (2) the distribution of comet types for each treatment group. Control group, (L1) LCT- treated group (20mg/kg), (L2) LCT- treated group (40mg/kg) (D1) DM - treated group (20mg/kg) (D2) DM - treated group(40mg/kg).

Table (4) statistical analysis compression among different treatment groups .

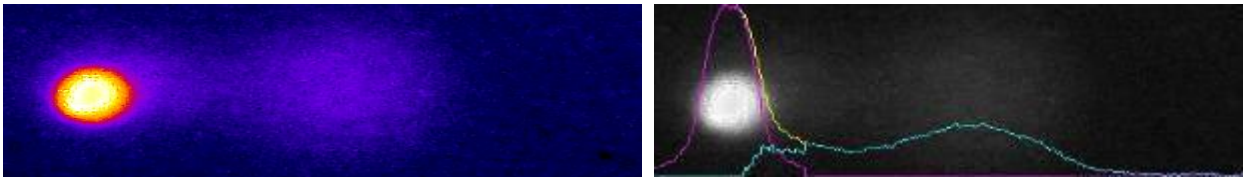
		Chi-square	DF	P value
	L1	413.82	4	0.000
	L2	433.16	4	0.000
	D1	400.77	4	0.000
Group(1)control	D2	486.02	4	0.000
Group(2) L1	L2	2.39	4	0.66
Group(4) D1	D2	2.96	4	0.56
Group(2) L1	D1	2.93	4	0.56
Group(3) L2	D2	13.26	4	0.01



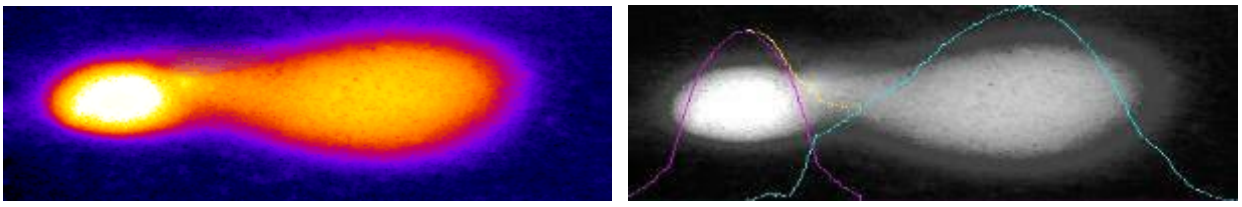
(**A**) undamaged cells (tailDNA% <5



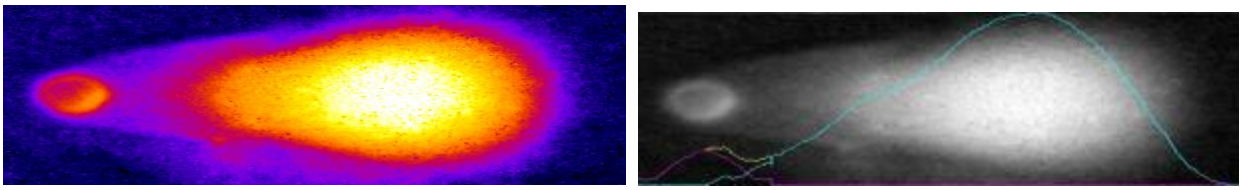
(**B**) low damaged cells (tail DNA% 5–25



(**C**) moderately damaged cells (tail DNA% 26–45



(**D**)highly damaged cells (tail DNA% 46–80



(**E**) extremely damaged cells (tail DNA% >80).

Figure (3) represent comet categories and analysis by comet score software , the left represent the actual comet , the right represent the analyzed comet (red trace for head DNA intensity , cyan trace for tail DNA intensity and yellow trace for overall DNA intensity).

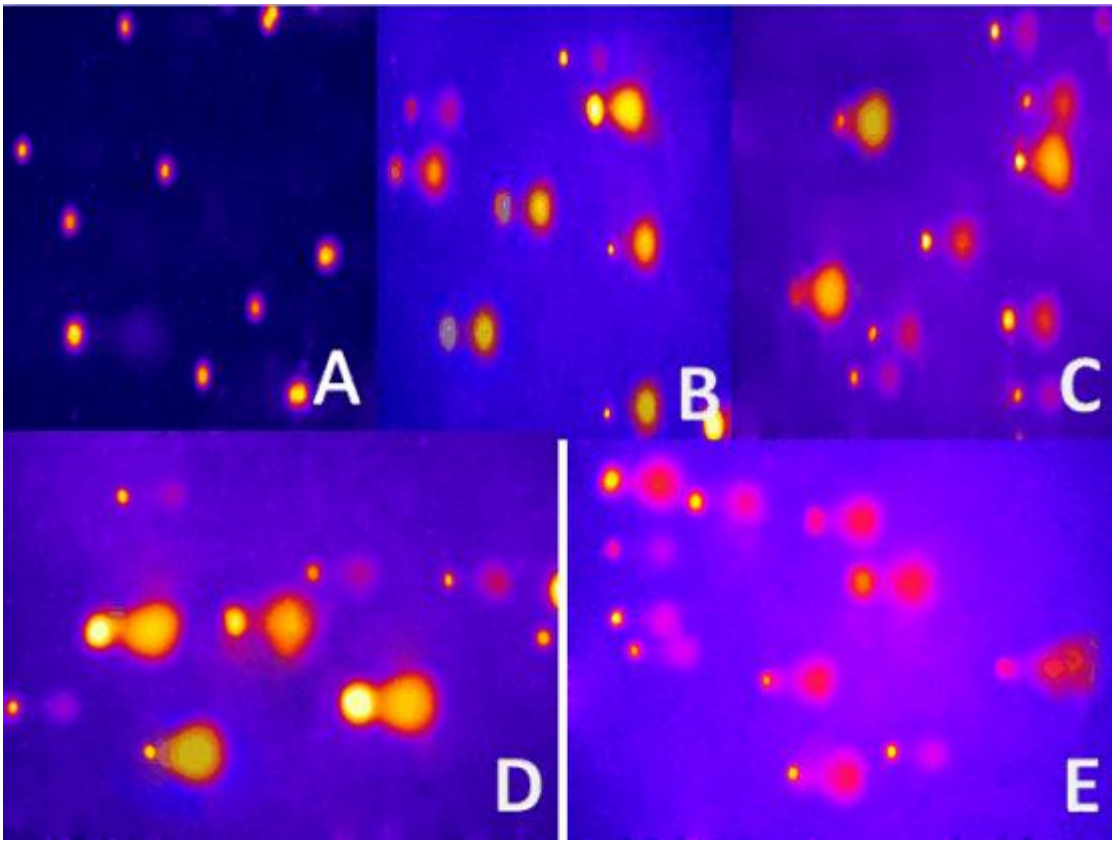


Figure (4): Photomicrographs representative the different degrees of DNA damage in the testes of male rats as evaluated by the comet assay after treatment by Lambada-cyhalothrin &dimethoate. (A) Control group, (B) LCT- treated group (20mg/kg), (C) LCT- treated group (40mg/kg) (D) DM - treated group(20mg/kg) (E) DM - treated group(40mg/kg).

3.4. Effect of Lambada-cyhalothrin &dimethoate on Testes tissue of adult male rats.

The effect of Lambada-cyhalothrin &dimethoate on testes cause tubular atrophy with loss of spermatogenesis, the effect grading as mild in cases loss of spermatogenesis 0-30% , moderate in loss 31-60%, severe loss 61-100%) (table5)

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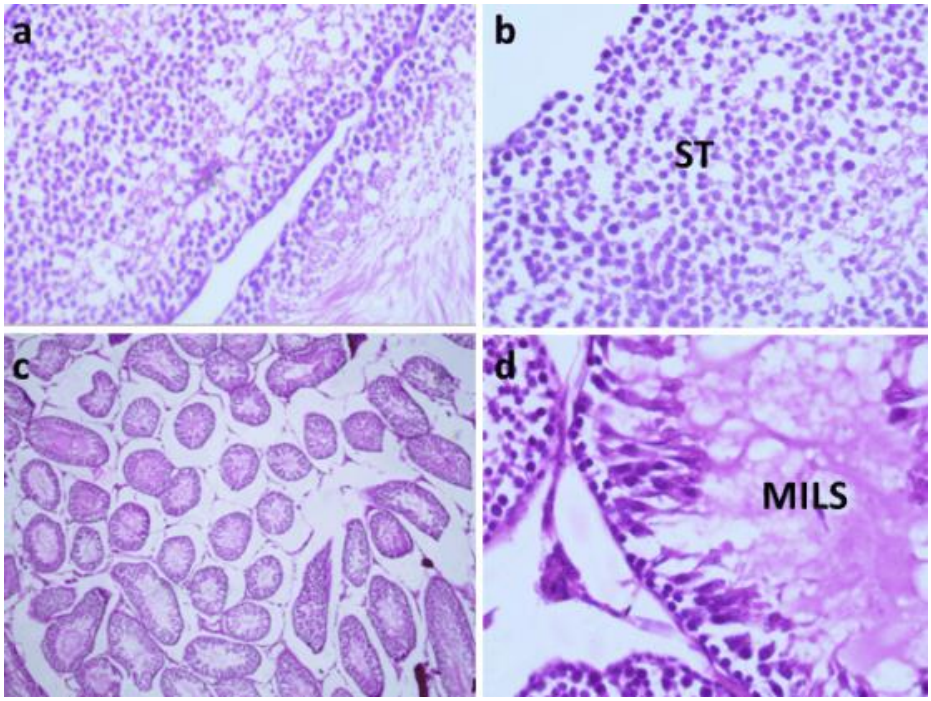
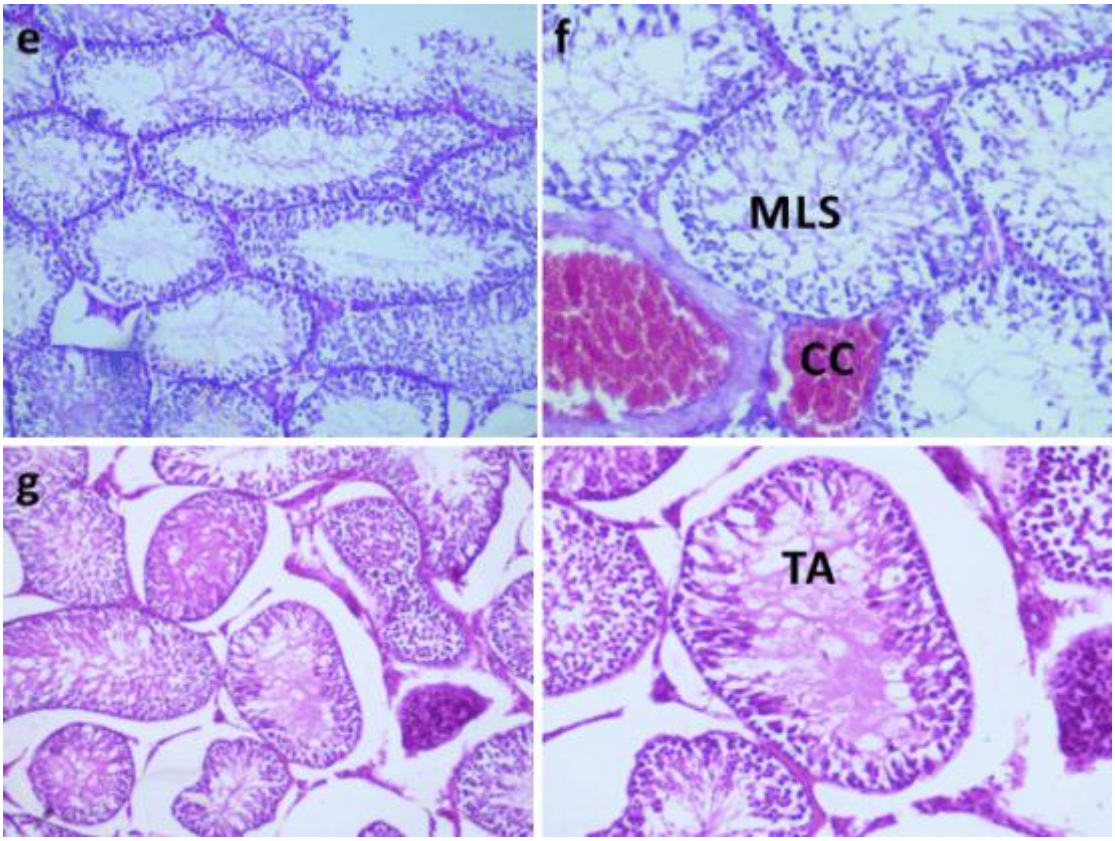
Table 5 : Effect of Lambada-cyhalothrin &dimethoate on Testes tissue samples of adult male rats.

Testes	Group1 Control	Group2 L1	Group3 L2	Group4 D1	Group5 D2	Total
	No. & %	No. & %	No. & %	No. & %	No. & %	No. & %
Normal	6 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (20.0)
Mild	0 (0.0)	6 (20.0)	4 (13.3)	5 (16.7)	1 (3.3)	16 (53.3)
Moderate	0 (0.0)	0 (0.0)	2 (6.7)	1 (3.3)	5 (16.7)	8 (26.7)

Total	6 (20.0)	6 (20.0)	6 (20.0)	6 (20.0)	6 (20.0)	30 (100%)
CalX^2 = 43.1 TabX^2 =15.51 DF= 8 P. value < 0.001 Significant						





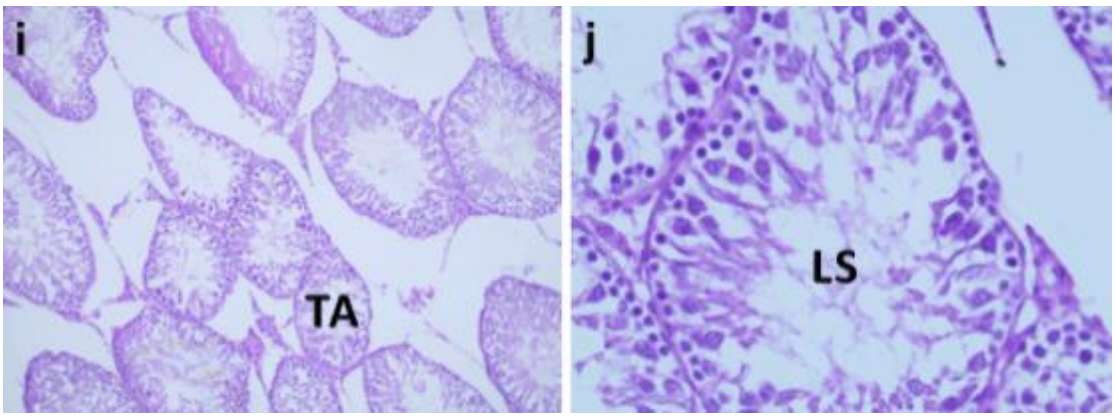


Fig (5)Light micrographs of rat testes tissue stained by hematoxylin-eosin (H&E) in control and treated groups. The control group, seminiferous tubules with no histological changes in germinal cell proportion and no edema in the interstitial tissue are present. The spermatogenesis are normal and seminiferous tubules with a high proportion of spermatozoa are observed(a, b) (×20,×40 respectively).Rats treated with lower dose (LCT) show mild loss of spermatogenesis(c, d) (×5,×40 respectively). Rats treated with higher dose (LCT) showmoderate loss of spermatogenesis with congested capillaries in stroma (e, f) (×10,×20 respectively). Rats treated with lower dose (DM) show tubular atrophy with edema of interstitium (g, h) (×10,×20 respectively). Rats treated with higher dose (DM) show

tubular atrophy with loss of spermatogenesis and mature sperms (i, j) (×10,×40 respectively).

ST seminiferous tubules,MILS mild loss of spermatogenesisMLSmoderate loss of spermatogenesis, CC congested capillaries, TA tubular atrophy, LS loss of spermatogenesis

4. Discussions

Exposure to organophosphorus pesticides was linked to a reduction in sperm concentration and motility[19].Male Rats treated with Lambada-cyhalothrin &dimethoate had lower sperm production and sperm motility as a percentage than the control group.

El-Hoda[20]showed that the studied pesticides' maximum concentrations significantly reduced sperm motility. However, the number of spermatozoa with morphological abnormalities increased and the sperm count dropped in treated rats at both concentrations of profenofos, diazinon, and chlorpyrifos methyl. According to Suresh and Preeti,[21]reproductive toxicity and a loss in both human and animal fertility have been connected to organophosphorous substances (organophosphates, OP). Male mice given lambda-cyhalothrin at various doses did not have fewer sperm, according to Ratnasooriya *et al.,*[22]After giving mice an organophosphate treatment, Aydogan and Barlas found that the percentages of abnormal sperm considerably increased in the treatment groups[23]. At both levels, every chemical that was examined increased the overall number of defective sperm. The most noticeable anomalies in sperm are typically twisted tails and coil tails with protoplasmic droplets. Lambda cyhalothrin exhibited the least amount of this effect, while the greatest concentrations of each pesticide reduced the sperm motility in treated rats.

All pesticides that were evaluated showed an increase in total sperm abnormalities at both doses. Typically, twisted tails and coiled tails with protoplasmic droplets are the most obvious abnormalities seen in sperm. The highest concentration of each pesticide caused a decrease in sperm motility in mice that had been treated, while lambdacyhalothrin caused the least amount of this effect. Even in the absence of any impact on fertility, sperm morphology and motility may serve as effective indicators of toxic harm. Male fertility is thought to be more accurately determined by sperm morphology than by sperm concentration. Sperm morphology and motility may act as reliable indications of toxic damage, even in the absence of any effect on fertility. Rather than sperm concentration, male fertility is separated from infertility by sperm morphology[24]. The current study's findings revealed an increase in sperm with abnormal morphology. Data

suggest a potential connection between exposure to studied pesticides and worse sperm quality. Additional evidence for testicular toxicity comes from studies in lab mice that linked pesticide exposure to irregular sperm shape as well as dose-response connections between exposure and declines in epididymal sperm count and motility and increases in defective sperm. Recent investigations have revealed two distinct routes through which pyrethroid-induced reproductive harm is also mediated, including direct cell effect and/or modification of vital metabolic events[25]. It was already known that the metabolites of the insecticide pyrethroid in human urine caused lower-quality sperm and more sperm DNA damage[26]. Similar effects of pyrethroids on animal reproductive have also been discussed in great detail. According to a variety of studies, the effect of pesticide on testis is connected to decreased sperm count, testosterone concentration, and fertility, as well as altered sperm characteristics. Additionally, LCT in male rats led to poor fertility and cell damage, according to Oshoke *et al.,*[27]. The impact of LCT on steroidogenesis and hormone levels, however, was unknown.Intricate neuroendocrine linkages control the secretion of testosterone (T), the main steroid sex hormone in male albino rats, via the testes' Leydig cells. Testosterone is required for the seminiferous tubules to continuously generate fresh generations of germ cells. Since testosterone is necessary for the male reproductive system, a disturbance in its synthesis could be harmful to the health of the male reproductive system. Lower testosterone levels in the LCT-treated group could cause the seminiferous tubule epithelium to separate from the germ cells[28].Low testosterone levels in LCT-treated rats may also be caused by decreased luteinizing hormone (LH), which is produced and released from the anterior pituitary by stimulation of gonadotropin-releasing hormone (GnRH) from the hypothalamus. LH controls how much testosterone and other androgens are produced by Leydig cells. Organophosphate is one of the most widely used synthetic pesticides[29].Organophosphates are widely used, which has prompted research into how exposure to them may affect both human and animal reproductive[30]. Therefore, it is critical for the public's health to ascertain the reproductive toxicity of organophosphorous insecticides. Dimethoate is a common organophosphate pesticide that is used extensively in both domestic and agricultural contexts. The results of the investigation showed that testosterone levels in the dimethoate-exposed rats considerably dropped. A very important hormone that is vital for the health of male reproductive organs is testosterone, which is produced by male testes. According to reports, testosterone levels have changed significantly in animals exposed to several organophosphate insecticides. These results imply that pesticides have a detrimental influence on sex hormones, particularly testosterone, which is crucial for the development of sperm[31].

The most obvious outcome of our comet analysis was that the Lambada-cyhalothrin & dimethoate treated group demonstrated a significant increase in the comet%, head diameter, tail length, DNA% tail, and tail moment in testicular tissues as compared to the control group. These findings indicate that DM is a genotoxic agent in the in vivo mammalian system[32-36]. Additionally, it is thought to be a cytotoxic stimulus that heightens oxidative stress and the production of ROS.Oxidative stress is assumed to be a combined pathogenic process that results in DNA damage and death in exposed rats since these radicals assault both proteins and DNA bases[37-40].

The testicular histology of rats exposed to of Lambada-cyhalothrin &dimethoate on testes cause tubular atrophy with loss of spermatogenesis, the loss of spermatogenesis cases grade the effect as modest.Dimethoate would cause cell death. This cytotoxic effect appears to be caused by a condition known as necrosis of the germ cells [41,42]. To reduce the incidence of environmental contamination, greater efforts must be taken to reduce the negative impacts of organophosphorus chemicals on the entire ecosystem. Therefore, we must be mindful of the harmful effects of Lambada-cyhalothrin & dimethoate on the male reproductive system, at least in elementary settings.

Conclusions

Although pesticides have been used for a very long period, it has only lately been discovered that male fertility has been declining, almost simultaneously with their rising usage. In light of this, despite the several probable explanations that have been mentioned, exposure to pesticides, particularly chronic exposure, should be viewed as the main contributor to the development of male infertility. This study explored the multiple negative effects that exposure to these chemicals had on the male reproductive system.

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