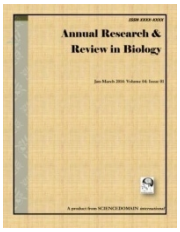

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Spermatogenic Alterations Induced by Organophosporus Compounds Profenofos, Chlorpyrifos and Synthetic Pyrethroid Lambada-cyhalothrin in Mice

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Authors' contributions

Author HME designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors SEN, AAS, MEK and FAHE managed the analyses of the study. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Background: Fertility is declining in many countries and there has been substantial interest in the potential adverse effects of exposure to environmental hazardous chemicals, including pesticides on male reproduction, and it was evident that using pesticides play as the major reasons for sperm decline besides tested pesticides are used widely recently, that is why it was very important the investigate their draw back in fertilization. The objective of the present study focuses on the spermiotoxicity of some pesticides such as profenofos, chlorpyrifos, and lambda-cyhalothrin on male albino mice.
Study Design: To assess the effect of tested pesticides on sperm morphology of male albino mice treated for 30, 60 and 90 consecutive days with different doses of pesticides (1/10, 1/40 and ADI LD_{50}).
Place and Duration of Study: Institute of animal health, Ministry of Agriculture, Egypt, between May 2011 and March 2012.
Results: Data suggest a potential association between exposures to tested pesticides and decreased sperm quality and increased teratospermic (abnormal sperm morphology).

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Further support for testicular toxicity comes from studies in laboratory albino mice that showed associations between exposure tested pesticides and sperm shape abnormalities, as well as dose-response relationships between exposure and a decline in epididymal sperm count and motility and increased abnormal sperm.
Conclusion: Tested pesticides can cause male reproductive system abnormalities that include reduced sperm production. It is also possible that the genetic information of the sperm may potentially be altered prior to fertilization.

Keywords: Male albino mice; lambada-cyhalothrin; profenofos; chlorpyrifos; sperm fertility; sperm motility; sperm shape abnormalities; primary spermatocytes.

1. INTRODUCTION

The health effects of pesticide exposures on male reproduction are a topic of considerable concern in environmental, occupational and reproductive epidemiology. In recent years, scientists have become more aware that human-made chemicals may disrupt reproductive function in wildlife and humans. Pesticides as human-made chemicals designed to kill living target organisms, are biologically active. An early insight into how pesticides can act as reproductive toxicants at the population level came from case reports in the 1970s of sterility among men working with the pesticides [1]. Human and animal data suggest a potential association between exposures to some commonly used insecticides and decreased sperm quality. Further support for testicular toxicity comes from studies in laboratory mice that showed associations between exposures tested pesticides and sperm shape abnormalities, as well as dose-response relationships between exposure and a decline in epididymal sperm count and motility and increased abnormal sperm morphology. Recently, the CDC reported that Chlorpyrifos increase sperm shape abnormalities of males in the United States [2]. Although both animal toxicology and human epidemiologic studies have shown that pesticides may operate through hormonal or genotoxic pathways to affect spermatogenesis. Profenofos considered as one of the male reproductive toxicants [3]. The objective of this investigation is to evaluate the effect of tested pesticides on sperm motility, morphology and primary spermatocytes in male albino mice, in order to recognize the effects of these insecticides to the environment and to determine the draw bakes of such chemicals on humans.

2. MATERIALS AND METHODS

2.1 Animals

80 male albino mice (aged 4-5 weeks, mean weight 20 gram) were used in this investigation. The animals were randomly housed in appropriate stainless cages in groups of 8 animals/cage. The animals were monitored daily for any abnormal symptoms prior to experimentation and weight changes were recorded weekly.

2.2 Chemicals

Lambda-cyhalothrin is a restricted synthetic pyrethroid insecticide. Profenofos, and Chlorpyrifos are organ phosphorus insecticides were kindly provided from Central Agricultural Pesticide Laboratory (Dokki, Giza, Egypt) and all compounds were of 99% purity.

2.3 Animal Treatment Schedule

Randomized groups of albino mice housed in cages containing saw dust as bedding and were allocated into 10 groups, each one contained 8 males, the first one group as a control, while the second, third, and fourth group were treated with Lambda-cyhalothrin at doses 1/10 LD_{50}, 1/40 LD_{50} and daily acceptable in take (ADI) for 30, 60, and 90 days respectively through oral administration by gavage. But the other groups were treated with Profenofos and Chlorpyrifos as a previously mentioned doses and period. Pesticides were given twice per weekly, as mentioned in Table (1).

Table 1. The treatment schedule and design

Treatment	Group No.	Doses mg/kg./b.wt	Period	Dose/weak
	Group (1)	**As a control**		
Lambda-cyhalothrin	Group (2)	1/10 LD_{50} = 9.5	30, 60, and 90 days	twice dose
	Group (3)	1/40 LD_{50} = 2.37		
	Group (4)	(ADI) = 0.005		
Profenofos	Group (5)	1/10 LD_{50} = 35	30, 60, and 90 days	twice dose
	Group (6)	1/40 LD_{50} = 8.95		
	Group (7)	(ADI) = 0.01		
Chlorpyrifos	Group (8)	1/10 LD_{50} = 15	30, 60, and 90 days	twice dose
	Group (9)	1/40 LD_{50} = 3.75		
	Group (10)	(ADI) = 0.01		

2.4 Sampling

The testes were removed by making an incision into the scrotum and fat tissue was cleaned as previously described in [4]. Then the tunica was removed and transferred into small petri dishes containing sodium citrate. The tunica was cut up with forceps several times, and then they were mashed on the fly mesh with flat-top forceps. The fluid containing the cells were transferred to 12 x 100 mm round bottom centrifuge tubes, centrifuged at 1000 r.p.m. for 5 min. Supernatant was completely discarded. The hypotonic solution (1% tris-sodium citrate) was slowly added and centrifuged, after 15-20 min., and then the cells were fixed in (methanol and glacial acetic acid, 3:1). The fixation was changed twice after 10 min., for each by centrifugation between changes.

2.5 Slide Preparation and Staining

Separated cells from tunica were transferred gently on slides then air dried. The slides were stained at least 10 min., using 10 % Giemsa (pH 6.8), washed and allowed to dry for subsequent light microscope analysis.

2.6 Sperm Analysis

Sperm motility and sperm morphological analysis was done according to the method described by [5].

3. RESULTS AND DISCUSSION

3.1 Analysis of Sperm Fertility, Measures and Abnormalities

Profenofos as well as Chlorpyrifos caused an increase in abnormal sperm heads and tails not only at all doses level used, but also at different time interval. Their frequencies in comparison with the control animals are shown in Table (2). Lambda-cyhalothrin caused fewer changes, these present evidence suggests that the percentage of abnormal sperms were affected by treatment doses and period. Various morphology sperm abnormalities were observed in control and treated animals Figs. (1-22). The most common types of abnormalities were amorphous, hook less and big head. Percentage of abnormal spermatozoa presented are shown in Table (2) and illustrated in Figs. (3-22).

Total sperm abnormalities were increased for all tested pesticides at both concentrations. Generally, the most pronounced malformations which were observed in sperms are bent tail, coiled tail with protoplasmic droplets. Sperm motility decreased in treated mice with each pesticide at the highest concentration and the least incidence was noticed with Lambda-cyhalothrin. Sperm morphology and motility could also be useful markers of toxic damage even in the absence of any effect on fertility. Sperm morphology is considered as a better discriminator between fertile and infertile males than sperm concentration [6].

The obtained results are in accordance with those found by [7], who revealed that Diazinon given orally to male rats for 65 consecutive days decreased sperm motility associated with an increase in the percentage of dead and morphologically abnormal spermatozoa. Methyl Parathion has been shown to induce reproductive abnormalities in both wild life and humans with reduction in sperm counts [8]. Furthermore, [9] found that Sub-lethal chronic administration (7-14 mg kg-1 a day for 15 days) of quinalphos resulted in severe disruption of spermatogenesis with increasing doses of pesticide. Remarkable reduction in the sperm count was observed in Westar rats following treatment with quinalphos (250 µg kg-1, i.p.) for approximately one (13 days) and two cycles (26 days) of the seminiferous epithelium [10]. Prior epidemiologic work on Chinese pesticide factory workers showed that organophosphorus pesticides exposure was associated with decreased sperm concentration and motility [11]. Sperm production and percentage of motile sperm were decreased in the 15 and 28 mg/kg/day treated male mice groups with Dimethoate compared to the control [12]. [13] showed that both the concentrations of the Chlorpyrifos methyl, Diazinon and Profenofos decreased sperm count associated with increase in the number of morphologically abnormal spermatozoa of treated rats; however sperm motility was significantly decreased with the highest concentration of the tested pesticides. [14] Mentioned that organophosphorous compounds (organophosphates, OP) are known to produce reproductive toxicity, decrease in the fertility levels of humans and animals.

These findings agree with [15] which reported that Cyhalothrin exposed to mice had a significantly smaller number of head dips in the whole board test. [16] Mentioned that male mice exposed to lambda-cyhalothrin in different doses had no effect on fertility. [17] Showed organophosphorus pesticides, are associated with male reproductive effects, including sperm chromatin alterations. [18] said that sperm counts and sperm morphology in the mice was decreased when exposed to Dichlorvos, also [19] found abnormalities in sperm density using Methyl parathion organophosphate changes such as epithelial cell morphology and luminal observations, the sperm density was normal in control, and moderately decreased in experiment 1 at 3.5 and 7 mg/kg. [20] Reported that mice treated with organophosphate it

has been observed that abnormal sperm percentages in treatment groups increased considerably.

The reduction in fertility index may simply represent the effects of Chlorpyrifos exposure on sperm parameters. Therefore, the effects of Chlorpyrifos on the fertility can be attributed to its ability to reduce sperm morphology and motility. Finally we cane concluded that both the concentrations of the tested pesticides decreased sperm motility associated with increase in the number of morphologically abnormal of treated mice; however sperm motility was significantly decreased with the highest concentration of the tested pesticides. Results showed that there was a correlation between Chlorpyrifos and Profenofos administration and the highly significant decrease of reproductive performance in male mice that agrees with [21].

The present study revealed that increased teratospermic (abnormal sperm morphology). Data suggest a potential association between exposures to tested used pesticides and decreased sperm quality. Further support for testicular toxicity comes from studies in laboratory mice that showed associations between exposure tested pesticides and sperm shape abnormalities, as well as dose–response relationships between exposure and a decline in epididymal sperm count and motility and increased abnormal sperm.

Finally, we can say that this is a preliminary work that shows some abnormalities in sperm structure, motility and nuclei morphology, and we suggest some important future studies, whole male reproductive organs sampled fertility tests must be done, to give a full picture of the caused male reproductive system abnormalities can be done using tested pesticides. It is also possible that the genetic information of the sperm may potentially be altered prior to fertilization. However, the evidence that such environmental chemicals cause infertility is still largely circumstantial. There are many missing links in the causal chain that would connect receptor binding to changes in reproductive health with decreased fertility.

Table 2. Effect on sperm morphology induced by Lambda-cyhalothrin, Profenofos, and Chlorpyrifos at (1/10, 1/40, LD_{50} and ADI) for 30, 60, and 90 days as respectively.

Pesticides	Doses	Period	Total abnormal sperm	Types of sperm abnormalities					
				Amorphous	Without book	Big head	Small head	Tail with 2 head	Others
Con.		30	6.2	1.4	1.5	1.1	0.8	0.5	0.9
		60	6.0	1.5	1.4	1.3	1.0	0.7	1.2
		90	7.2	1.8	1.7	1.2	1.1	1.0	1.5
Lamba.	1/10	30	13.5	3.2	2.7	1.9	1.7	1.5	2.5
		60	15.3	3.5	3.0	2.3	1.8	1.9	2.8
		90	16.5	3.8	3.2	2.4	2.0	2.1	3.0
	1/40	30	15.0	2.9	3.0	2.6	2.0	1.7	2.8
		60	17.1	4.1	3.1	2.7	2.4	1.9	2.9
		90	18.4	4.2	3.5	2.9	2.5	2.2	3.1
	ADI	30	10.5	1.7	1.0	1.2	1.3	1.4	1.3
		60	8.3	1.5	1.3	1.2	1.4	1.4	1.5
		90	8.8	1.8	1.4	1.3	1.2	1.6	1.5
Profenofos	1/10	30	18.2	5.1	3.4	2.5	1.9	2.3	3.0
		60	20.9	5.8	3.7	2.8	2.5	2.7	3.4
		90	25.6	7.1	4.4	3.5	2.7	3.2	4.7
	1/40	30	18.9	4.6	3.5	3.1	2.2	2.1	3.4
		60	21.9	5.1	4.2	3.7	2.5	2.8	3.6
		90	23.9	5.5	4.6	4.1	3.1	3.1	3.5
	ADI	30	10.8	2.5	1.9	1.4	1.7	1.5	1.8
		60	11.6	2.7	2.2	1.4	1.6	1.8	1.9
		90	12.3	2.8	2.0	1.7	2.0	1.9	1.9
Chlorpyrfos	1/10	30	18	4.8	3.3	2.4	1.9	2.4	3.2
		60	18.3	5.5	3.9	2.9	2.5	2.8	3.7
		90	25.4	6.1	4.6	3.8	3.3	3.5	4.1
	1/40	30	19.9	5.4	3.6	2.3	2.7	2.1	3.8
		60	23.4	5.9	4.7	3.0	2.9	2.8	4.1
		90	26.9	6.5	5.4	3.2	3.7	3.4	4.7
	ADI	30	10.8	1.9	2.0	1.6	1.6	1.9	1.8
		60	11.5	2.0	2.1	1.6	1.7	2.1	2.0
		90	12.2	2.2	2.2	1.9	1.7	2.2	2.0

100 cells were counted

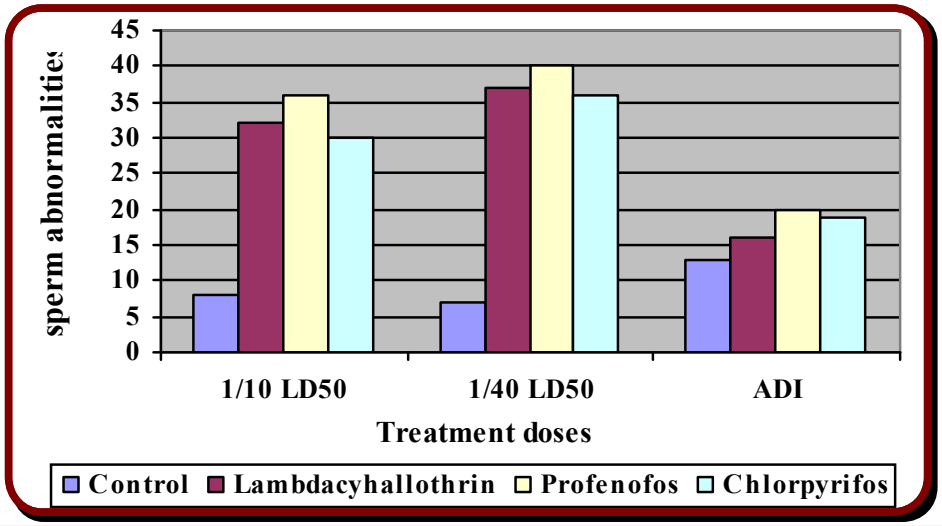

Fig. 1. Changes in sperm shape after treatment with tested pesticides

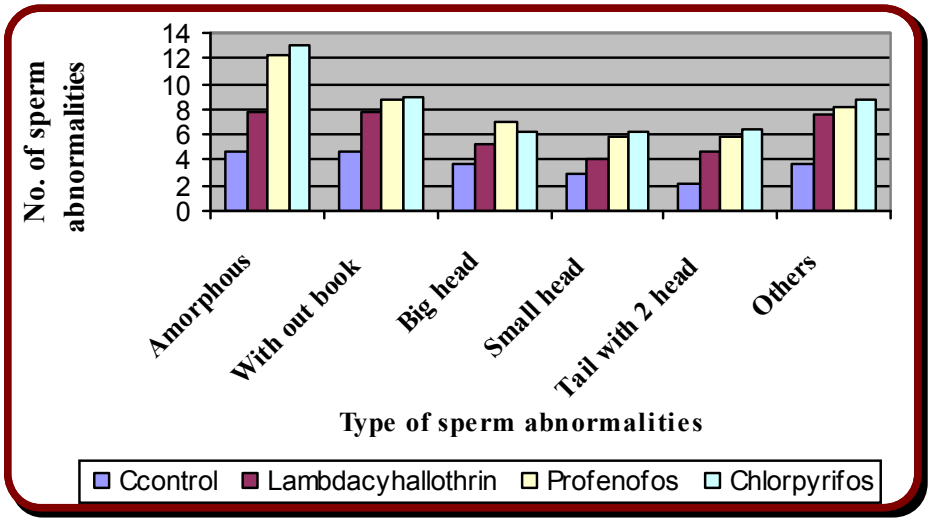

Fig. 2. Type of changes in sperm shape after treatment with tested pesticides

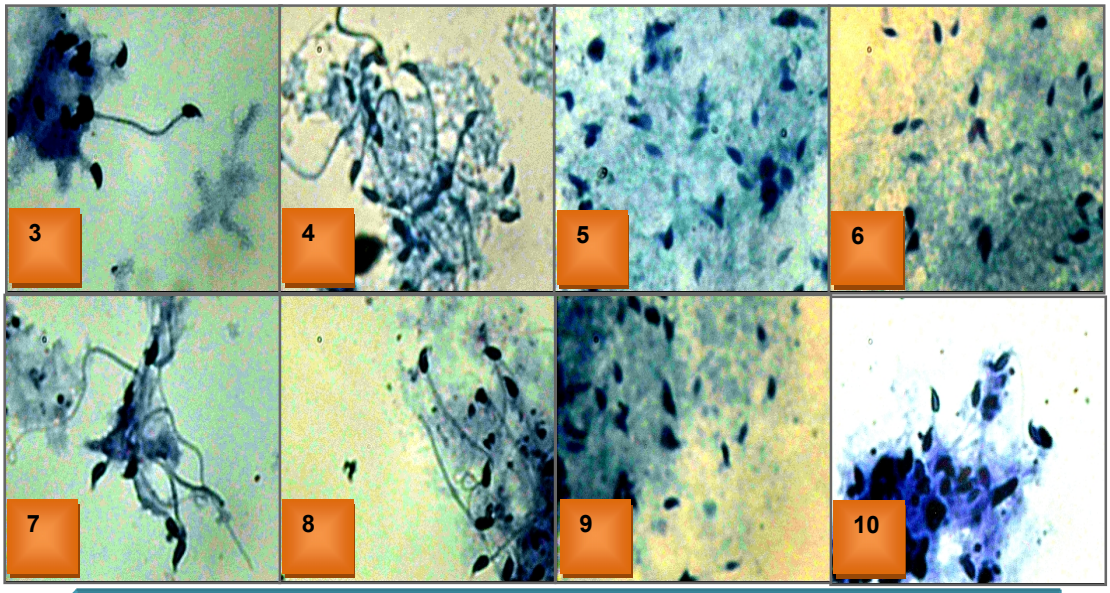

Fig. (3,4): Photomicrograph of mice sperm morphology as a negative control.
Fig. (5,6): Photomicrograph of mice sperm morphology induced by lambda-cyhalothrin at (1/10 LD_{50}) for 90 days.
Fig. (7,8): Photomicrograph of mice sperm morphology induced by lambda-cyhalothrin at (1/40 LD_{50}) for 90 days.
Fig. (9,10): Photomicrograph of mice sperm morphology induced by profenofos at (ADI).

(Using 10 % Giemsa, and X 1000)

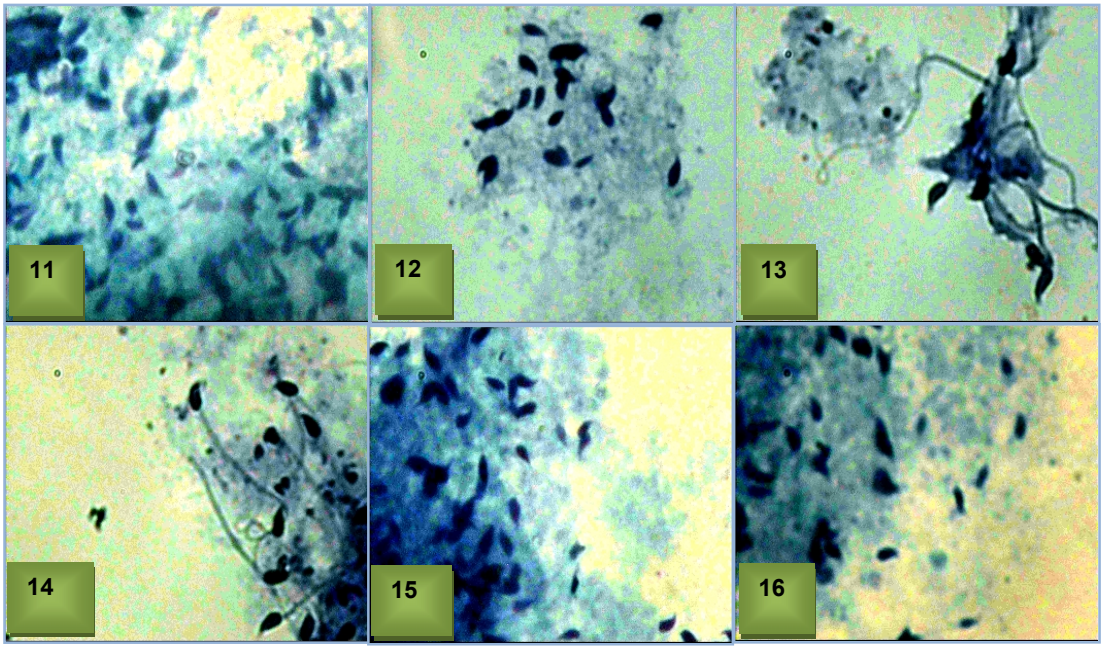

Fig. (11,12): Photomicrograph of mice sperm morphology induced by pofenofos at (1/10 LD_{50}) for 90 days.
Fig. (13,14): Photomicrograph of mice sperm morphology induced by pofenofos at (1/40 LD_{50}) for 90 days.
ig. (15,16): Photomicrograph of mice sperm morphology induced by profenofos at (ADI) for 90 days.

(Using 10 % Giemsa, and X 1000)

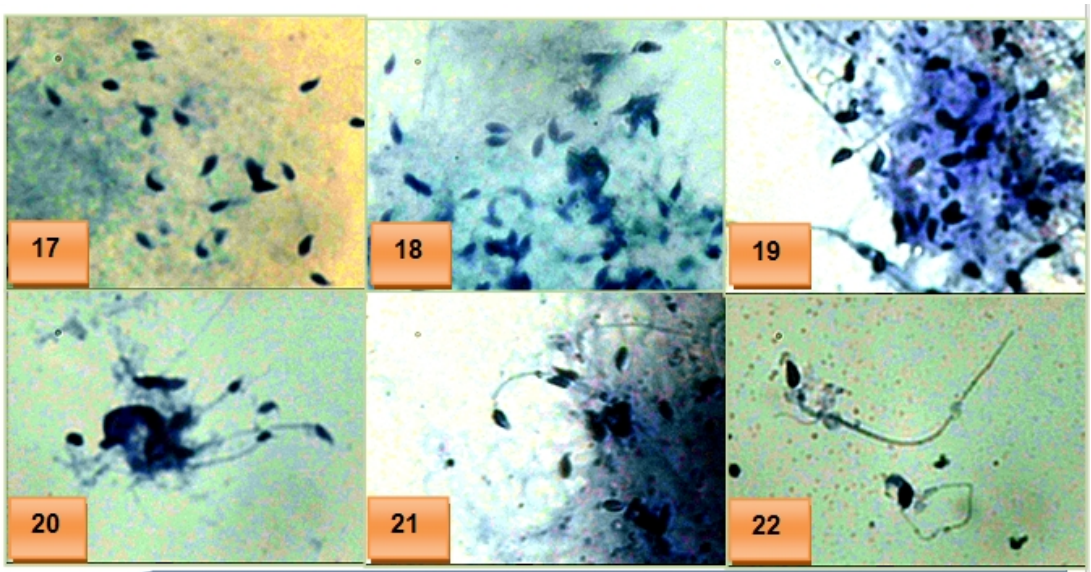

Fig. (17,18): Photomicrograph of mice sperm morphology induced by chloropyrifos at (1/10 LD_{50}) for 90 days. (stain?X 1000)
Fig. (19,20): Photomicrograph of mice sperm morphology induced by chloropyrifos at (1/40 LD_{50}) for 90 days. (stain?X 1000)
Fig. (21,22): Photomicrograph of mice sperm morphology induced by chloropyrifos at (ADI) for 90 days. (stain?X 1000)

(Using 10 % Giemsa, and X 1000)

3.2 Analysis of Mice Primary Spermatocytes

The results obtained from the analysis of diakinesis stage in mice primary spermatocytes after treatment with the Lambda-cyhalothrin, Profenofos and Chlorpyrifos is illustrated in Table (3). Three different types of aberration were observed they are stickiness, exchanges, and univalent of se as well as of autosomal chromosomes were observed in Figs (23-44). After treatment with tested pesticides stickiness ranged from 4, 4, and 5 in the negative control to 9, 13, and 14 after treatment with the highest tested dose 1/10 LD_{50} for 90 days with the Lambda-cyhalothrin, Profenofos and Chlorpyrifos as respectively. Univalent involved X, Y and autosomal chromosomes were obtained. The total percent of aberrant cells ranged from 8 to 13% for the control group. Meanwhile, Chlorpyrifos highly significantly decreased by 39, 67, and 19 after treatment with 1/10 LD_{50}, and 1/40 LD_{50} and (ADI) for 90 days respectively. In similar, Profenofos caused significant decrease after treatment with 1/10 LD_{50}, 1/40 LD_{50} and (ADI) as recorded 66, 63, and 17 for 90 days respectively. Also, Lambda-cyhalothrin caused decrease after treatment with 1/10 LD_{50}, 1/40 LD_{50} and (ADI) as 40, 66, and 23 for 90 days respectively.

It was found that the tested pesticides were capable to cause univalent X, Y as well as autosomal chromosomes. Illustrates stickiness and univalents obtained after treatment with all tested pesticides. Cytological examination proved that in the control group binucleat and multinuclei were not observed. At a low dose level 1/40 the binucleat cells were shown to be 20, 19 and 18 and multinuclear were 19, 18, and 18, while the higher dose 1/10 binucleat

cells were estimated to be 18, 22 and 20 and multinuclei were 16, 17, and 16 as well treated with Lambda-cyhalothrin, Profenofos, and Chlorpyrifos for 90 days respectively.

The data revealed that significant decreased of fertility after administration of all tested pesticides either in high (1/10 LD_{50}) or low dose (1/40 LD_{50}) within the three post treatment period (30, 60 and 90 days) respectively. In the similar effect between high dose (1/10 LD_{50}) and low dose (1/40 LD_{50}), while with (ADI) dose the result showed no significant changes with all tested pesticides and all treatment period. Profenofos was proven to induce different types of aberration in mice germinal cells more than Lambda-cyhalothrin, and Chlorpyrifos.

Chlorpyrifos administration of (1, 10 and 100 mg/kg b.w./day) to mature mice (F0) through pre-mating, mating, gestation and lactation period and to their offspring (F1) until 13 weeks age via gavages, its caused decreased in fertility index and numbers of implantation and born pups and a higher male sex ratio of pups.

This finding disagree with [22] which reported that Dimethoate was given orally by gavage to male mice for 20 days before mating with untreated females the percent morphologically normal spermatozoa were unaffected in any of dose groups however, sperm production and percent motile sperm were decreased in the 15 and 28 mg/kg/day treated groups compared to the control. On the other hand [23] reported male mice were exposed to Methyl parathion (20 mg/kg bw,i.p.) and spermatozoa from epididymis-vas deferens were collected at 7 or 28 days post-treatment to assess the effects on maturing spermatozoa and spermatocytes, respectively, in spermatozoa collected at 7 and 28 (dpt), and decreases in sperm quality and induced acrosome reactions were observed; reduced mitochondrial membrane potential and lipoperoxidation were observed at 7 (dpt) only.

However [24] studied the effect of Endosulfan on bluegill testes after 24 h of exposure there was evidence of slight signs of connective tissue splintering, after 48-h exposure resulted in breakage of primary spermatocyte walls and separation from the seminiferous tubules but after 72-h testis showed further connective tissue damage and migration of primary spermatogonia into the lumen, after 96 h, there was significant damage to connective tissue and the seminiferous tubules were less pronounced, after 1 and 2 weeks, the seminiferous tubule walls were disrupted and missing in places and the structure of the testis was much disorganized compared to the control testis, biometric analysis indicated that the diameter of the primary spermatogonia decreased from 24 h to two weeks, these kinds of damage could affect the spermatids and spermatozoa and possibly have a negative impact on spermatogenesis and male fertility. Finally our results showed that decrease in concentrations of spermatozoaas the same described with [25]. The same with [26] which reported that Diazinon causes the damage of the germinal epithelium in the testes leading to the spermatogenesis failure, damaged and separating spermatids lines, reduced spermatogenesis. Also [27] mentioned that cadmium and Diazinon exerted deleterious effect inducing spermatozoa motility alterations which could be subsequently negatively related to male fertility.

Table 3. Effect on mice primary spermatocytes induced by Lambda-cyhalothrin, Profenofos, and Chlorpyrifos at (1/10, 1/40, from LD_{50} and ADI) for 30, 60, and 90 as respectively

Pesticides	Doses	Period	Stickiness	Univalent				
				XY	Autosomes	Binucleate	Multinuclear	Total percent of aberant cells
Cont.		30	4.0	2	2	0	0	8
		60	4.0	2	1	0	0	7
		90	5.0	3	2	1	2	13
Lamba-	1/10	30	5.0	4	2	11	10	32
		60	8.0	5	5	15	13	35
		90	9.0	5	7	18	16	40
	1/40	30	6.0	4	3	13	11	51
		60	8.0	6	6	15	16	40
		90	12.0	7	8	20	19	66
	ADI	30	4.0	3	2	4	3	16
		60	4.0	3	2	5	3	17
		90	5.0	4	3	6	5	23
Profenofos	1/10	30	5.0	3	3	14	11	36
		60	8.0	5	6	18	15	52
		90	13.0	6	8	22	17	66
	1/40	30	5.0	4	3	15	13	40
		60	7.0	6	5	17	14	49
		90	10.0	7	9	19	18	63
	ADI	30	3.0	2	2	5	4	16
		60	5.0	2	2	5	5	16
		90	4.0	3	2	6	5	17
Chlorpyrifos	1/10	30	7.0	4	4	15	10	55
		60	11.0	6	6	17	15	39
		90	14.0	7	7	20	16	39
	1/40	30	7.0	4	3	13	12	36
		60	13.0	5	5	16	16	55
		90	17.0	7	7	18	18	67
	ADI	30	4.0	2	2	5	3	16
		60	3.0	1	2	4	3	13
		90	5.0	2	3	5	4	19

100 cells were counted

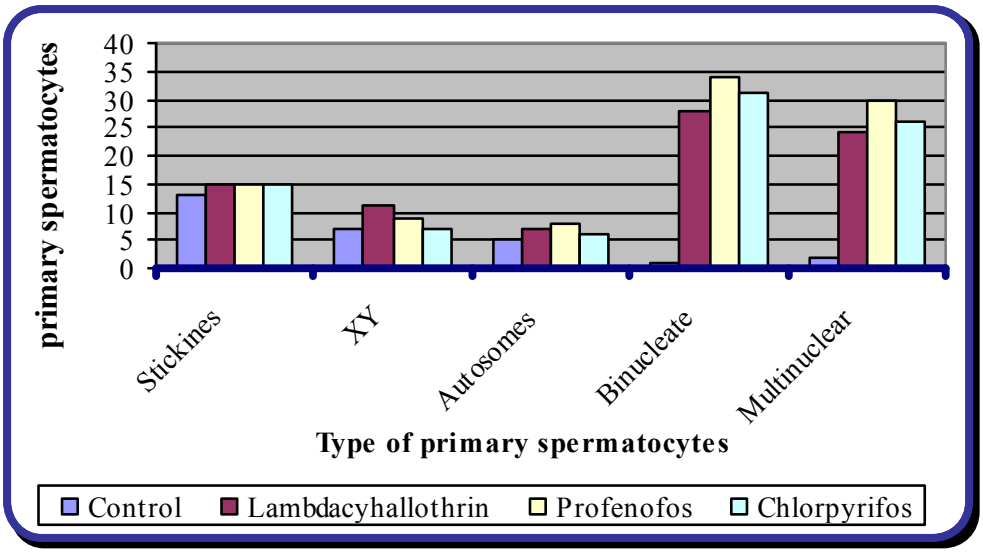

Fig. 23. Type of changes in mice primary spermatocytes aberrations after treatment with tested pesticides

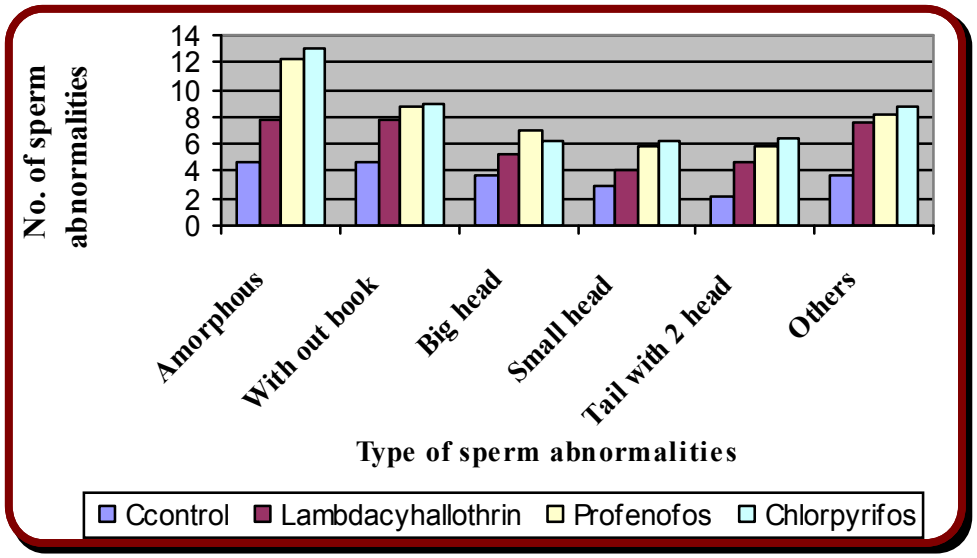

Fig. 24. Type of changes in sperm aberrations after treatment with tested pesticides

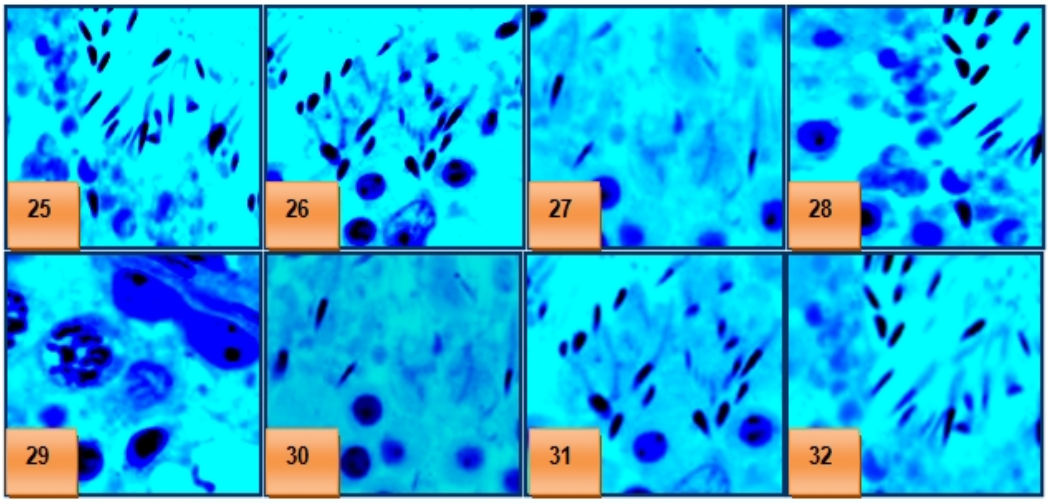

Fig. (25,26): Photomicrograph of mice primary spermatocytes aberrations as a negative control.
Fig. (27,28): Photomicrograph of mice primary spermatocytes aberrations induced by ambda-cyhalothrin at (1/10 LD_{50}) for 90 days.
Fig. (29,30): Photomicrograph of mice primary spermatocytes aberrations induced by lambda-cyhalothrin at (1/40 LD_{50}) for 90 days.
Fig. (31,32): Photomicrograph of mice primary spermatocytes aberrations induced by profenofos at (ADI).

(Using 10 % Giemsa, and X 1000)

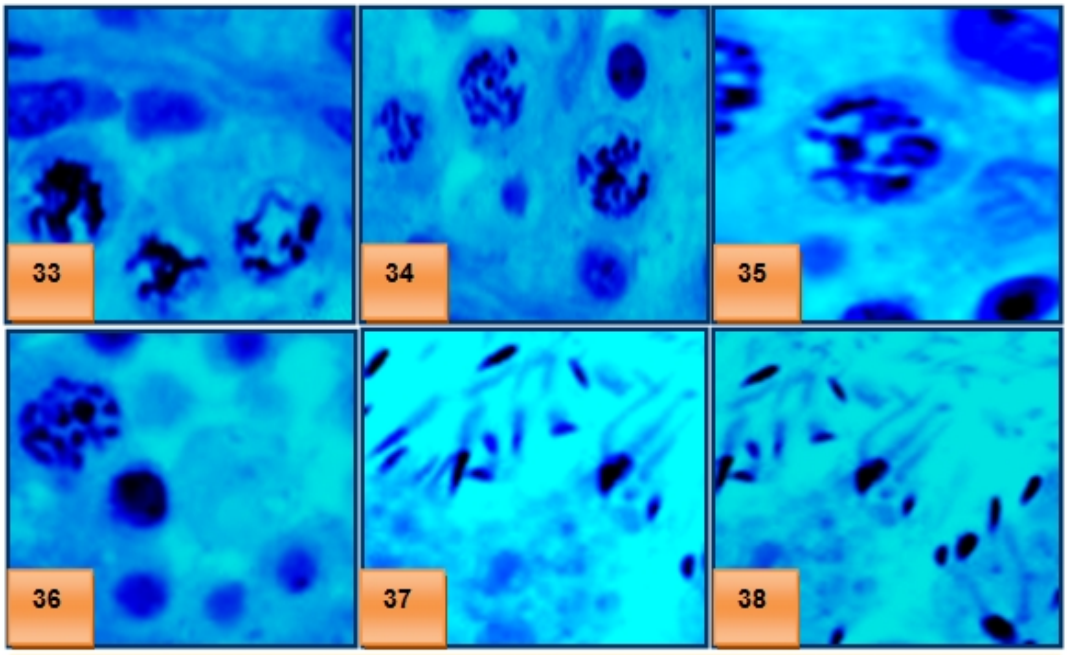

Fig. (33,34): Photomicrograph of mice primary spermatocytes aberrations induced by pofenofos at (1/10 LD_{50}) for 90 days.
Fig. (35,36): Photomicrograph of mice primary spermatocytes aberrations induced by pofenofos at (1/40 LD_{50}) for 90 days.
Fig. (37,38): Photomicrograph of mice primary spermatocytes aberrations induced by profenofos at (ADI) for 90 days.

(Using 10 % Giemsa, and X 1000)

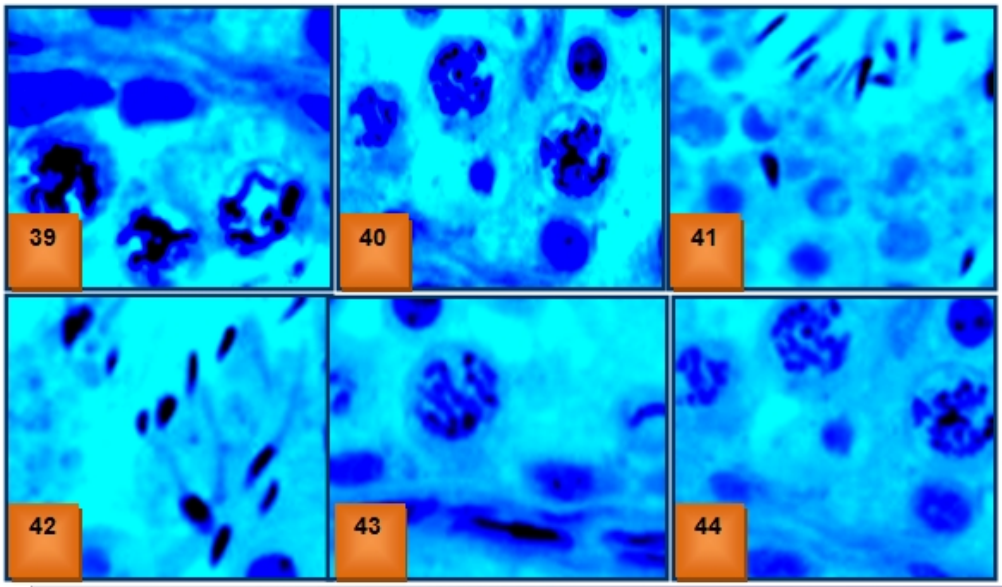

Fig. (39,40): Photomicrograph of mice primary spermatocytes aberrations induced by chloropyrifos at (1/10 LD_{50}) for 90 days.
Fig. (41,42): Photomicrograph of mice primary spermatocytes aberrations induced by chloropyrifos at (1/40 LD_{50}) for 90 days.
Fig. (43,44): Photomicrograph of mice primary spermatocytes aberrations induced by chloropyrifos at (ADI LD_{50}) for 90 days.

(Using 10 % Giemsa, and X 1000)

4. CONCLUSION

The results obtained have shown that sperm abnormalities increased in treated mice with all tested pesticides at both concentrations. Therefore, we suggest that these pesticides should be used at recommended doses only if necessary.

COMPETING INTERESTS

Authors have declared that no competing interests exist

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