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

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ABSTRACT

Aims: 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. Organophosphorous and pyrethroid compounds are known to produce reproductive toxicity. Reduced fertility in males is one of the major end points of reproductive toxicity, so the objective of the present study mainly focused on toxicity of some pesticides such as profenofos, chlorpyrifos, and lambda-cyhalothrin especially dealing with reproductive toxicity in males (sperm motility, sperm shape abnormalities, and primary spermatocytes 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). 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 and/or fertilizing capability. It is also possible that the genetic information of the sperm may potentially be altered prior to fertilization.

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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 Teitelbaum, (1999). 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, CDC (2003). 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 Moustafa *et al.,* (2007).The objective of this investigation is to evaluate the effect of tested pesticides on sperm fertility, sperm motility, sperm shape abnormalities, and primary spermatocytes in male albino mice, in order to recognize the computability of these insecticides to the environment and to determine the draw bakes of such chemicals on humans.

2.1. Animals: 80 male albino mice were used in this investigation, aged 4-5 weeks and of mean weight 20 gram. The animals were randomly housed in appropriate stainless cages in groups of 8 animals/cage. The animals were rearranged to group; they were also monitored daily for abnormal symptom and weight change was recorded weekly.

2.2. Chemicals: Lambda-cyhalothrin: is a restricted use synthetic pyrethroid insecticide. The active ingredient (Lambda-cyhalothrin 99.8 % Agrochemical Co.). Profenofos, and Chlorpyrifos are an organophosphorus insecticides. Commercially 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 the oral administration. 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 Alder (1984). Then, the tunica was removed and transferred into small Petri dishes containing sodium citrate. The tubes were 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 were transferred gently on slides then air dried. The slides were stained at least 10 min., using 10 % Giemsa (pH 6.8) or orcein, washed and allowed to dry for subsequent light microscope analysis.

2.6. Sperm analysis: Sperm motility and sperm morphological analysis according to the method described by Jeong *et al.,* (2005).

3. RESULTS AND DISCUSSION

3.1. Analysis of sperm fertility, measures and abnormalities:

Various morphology sperm abnormalities Fig (1-22) were observed in control and treated animals. The most common types of abnormalities were amorphous, hookless and big head. Percentage of abnormal spermatozoa is present in Table (2) and illustrated in Fig (3-22). 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 Table (1). Lambda-cyhalothrin caused less changes. These present evidence suggests that the percentages of abnormal sperms were affected by treatment doses and period.

The percentages of sperm motility decreased in treated mice with each pesticide at the highest concentration and the least incidence was noticed with lambda-cyhalothrin. 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 and protoplasmic droplets. The abnormalities appeared as bent tail, constitute the highest percentages of the total deformities. Sperm morphology is considered as a better

discriminator between fertile and infertile males than sperm concentration Guzick *et al.,* (2001). Sperm morphology and motility could also be useful markers of toxic damage even in the absence of any effect on fertility.

The obtained results are in accordance with those found by Abd El-Aziz *et al.* (1994), 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 Mathew *et al.,* (1992). Furthermore, Sarkar (2000) 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 Wistar 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 Ray *et al.,* (1992). Prior epidemiologic work on Chinese pesticide factory workers showed that organophosphorus pesticides exposure was associated with decreased sperm concentration and motility Padungtod *et al.,* (2000). 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 Farag *et al.,* (2007). El-Hoda A. Zidan (2009) showed that both the concentrations of the chlorpyrifos methyl, diazinon and profenofos decreased sperm count associated with increase in the number ofmorphologically abnormal spermatozoa of treated rats; however sperm motility was significantly decreased with the highest concentration of the tested pesticides. Suresh C. Joshi and Preeti Sharma (2011) 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 Silva Gomes, (1991) which reported that cyhalothrin exposed to mice had a significantly smaller number of head dips in the whole board test. Ratnasooriya *et al.*, (2002) mentioned that male mice exposed to lambda-cyhalothrin in different doses had no effect on fertility. Piña-Guzmán B. *et al.,* (2005) showed organophosphorus pesticides, are associated with male reproductive effects, including sperm chromatin alterations. Ai Okamura *et al.,* (2005) said that sperm counts and sperm morphology in the mice was decreased when eexposed to Dichlorvos, also Narayana K. *et al.,* (2006) 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. Aydogan M., and Barlas N., (2006) reported that mice treated with organophosphate it has been observed that abnormal sperm percentages in treatment groups increased considerably.

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 Ahmed *et al.,* (2012). 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.

Data suggest a potential association between exposures to tested used pesticides and decreased sperm quality. The present study revealed that increased teratospermic (abnormal sperm morphology). 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.

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

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.

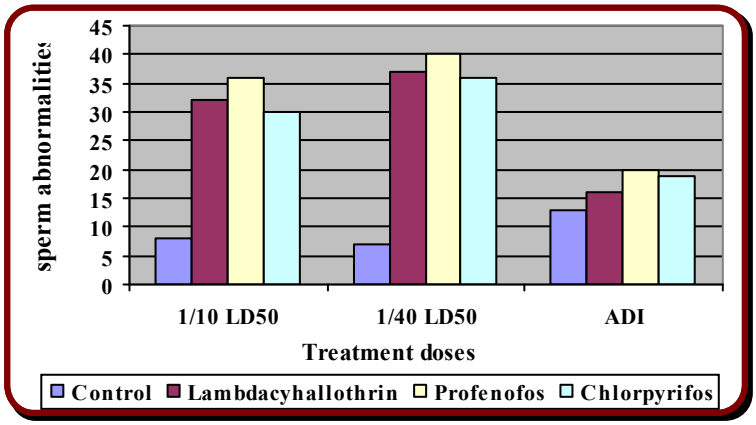


Fig. (1): Changes in sperm aberrations after treatment with tested pesticides

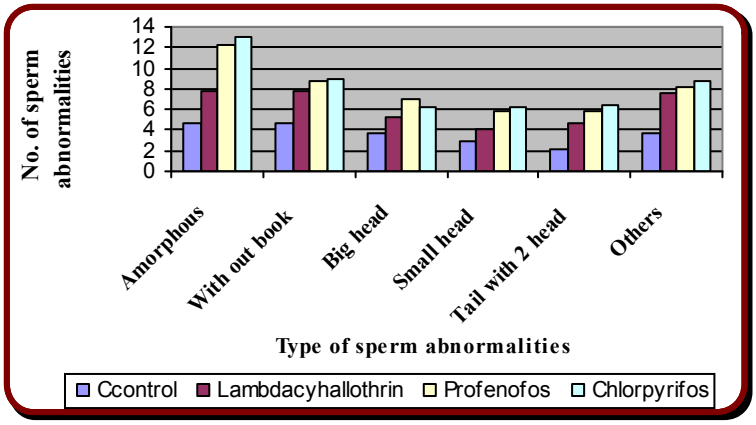
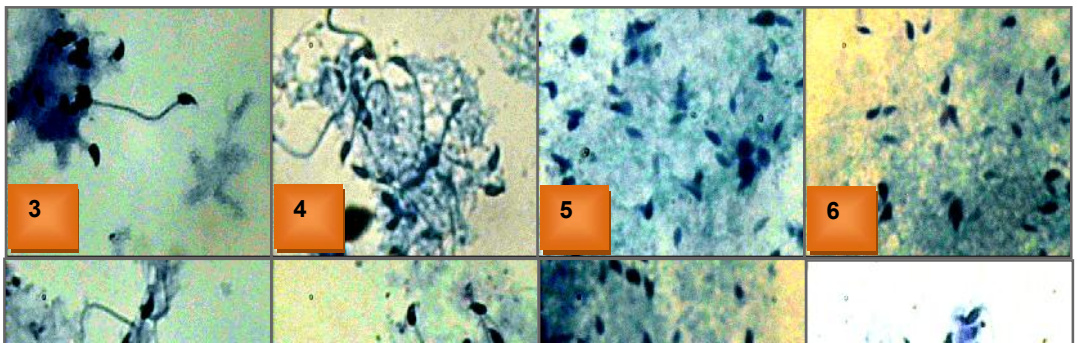


Fig. (2): Type of changes in sperm aberrations after treatment with tested pesticides



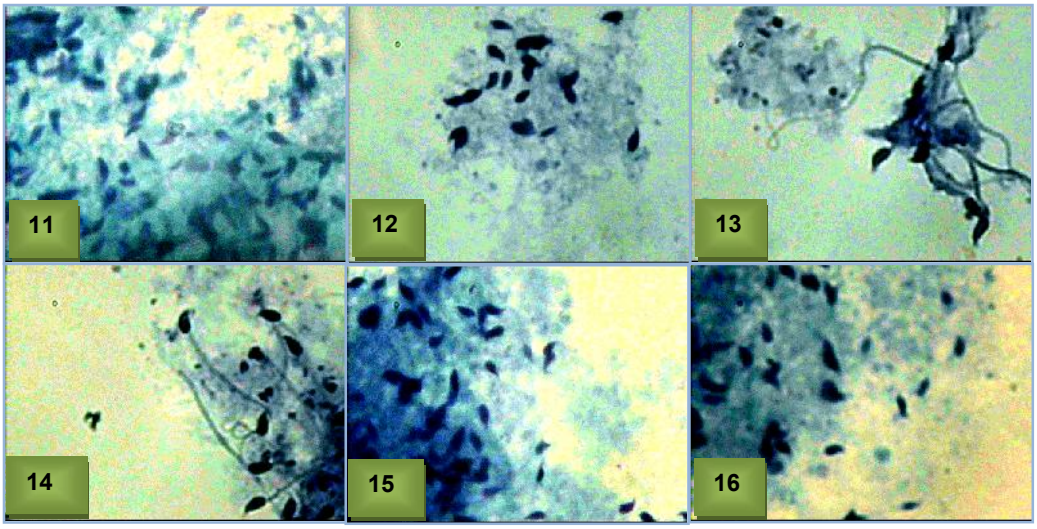
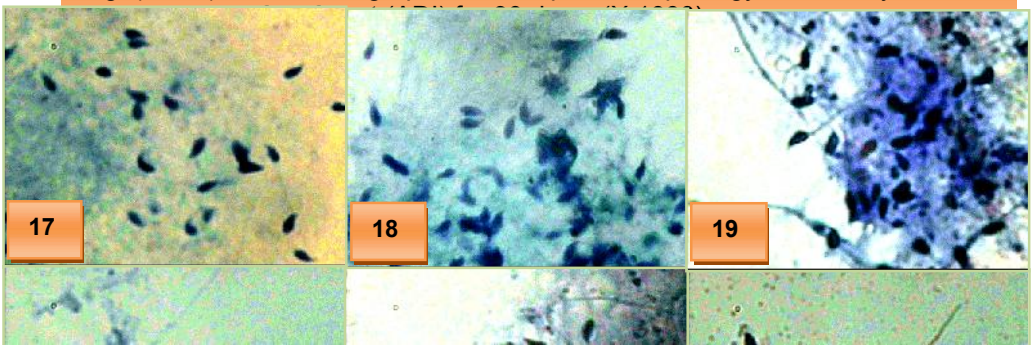


Fig. (11,12): Photomicrograph of mice sperm morphology induced by pofenofos at (1/10 LD_{50}) for 90 days. (X 1000)
Fig. (13,14): Photomicrograph of mice sperm morphology induced by pofenofos at (1/40 LD_{50}) for 90 days. (X 1000)
Fig. (15,16): Photomicrograph of mice sperm morphology induced by



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 Fig. (3-22). 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 multinuclei 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 hight (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 Amina *et al.,* (2007) 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 Piña-Guzmán *et al*., (2009) 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 Dutta *et al.,* (2006) 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 Muftau *et al.,* (2013). The same with Michal *et al.,* 2010 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 Maria *et al.,* (2012) 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

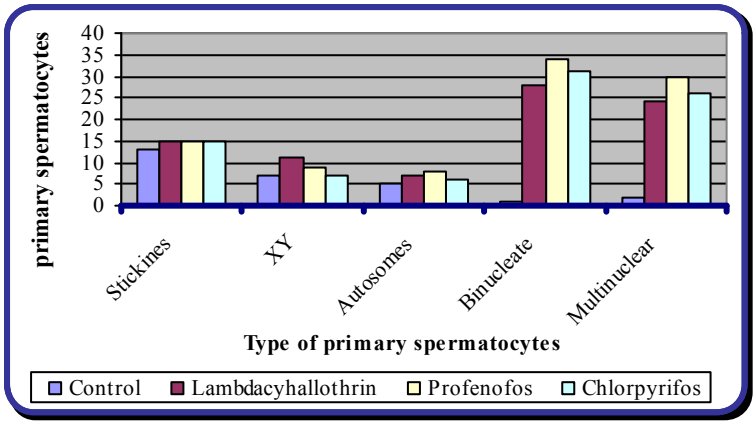


Fig. (1): Type of changes in mice primary spermatocytes aberrations after treatment with tested pesticides.

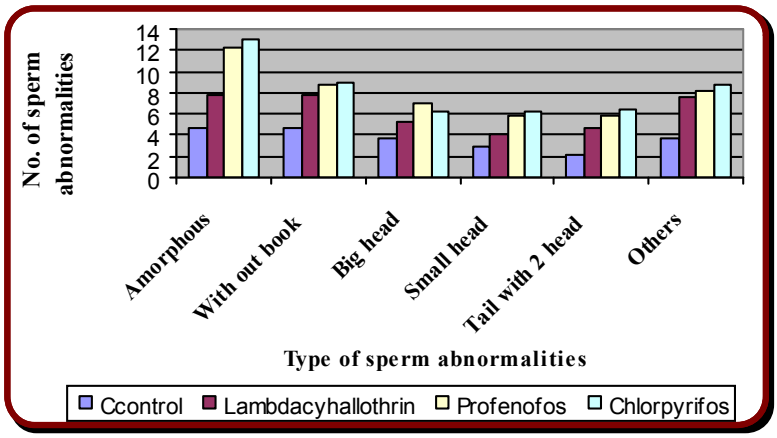


Fig. (2): Type of changes in sperm aberrations after treatment with tested pesticides.

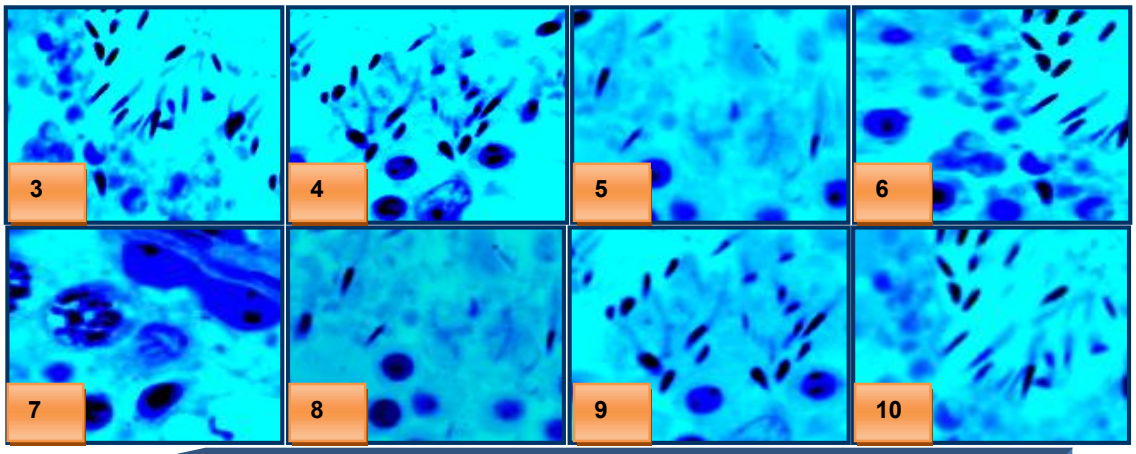


Fig. (3,4): Photomicrograph of mice primary spermatocytes aberrations as a negative control. (X 1000)
Fig. (5,6): Photomicrograph of mice primary spermatocytes aberrations induced by ambda-cyhalothrin at (1/10 LD_{50}) for 90 days. (X 1000)
Fig. (7,8): Photomicrograph of mice primary spermatocytes aberrations induced by lambda-cyhalothrin at (1/40 LD_{50}) for 90 days. (X 1000)
Fig. (9,10): Photomicrograph of mice primary spermatocytes aberrations induced by profenofos at (ADI). (X 1000)

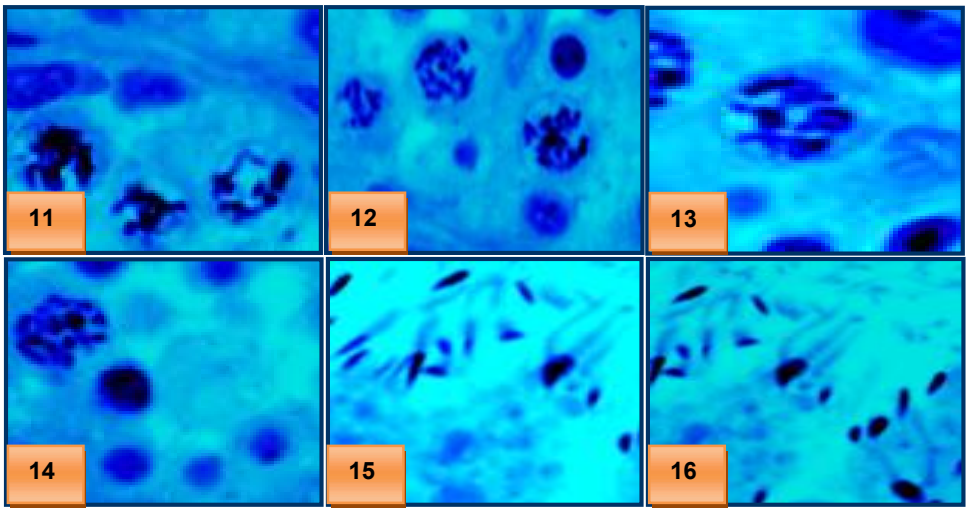


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Fig. (15,16): Photomicrograph of mice primary spermatocytes aberrations induced by profenofos at (ADI) for 90 days. (X 1000)

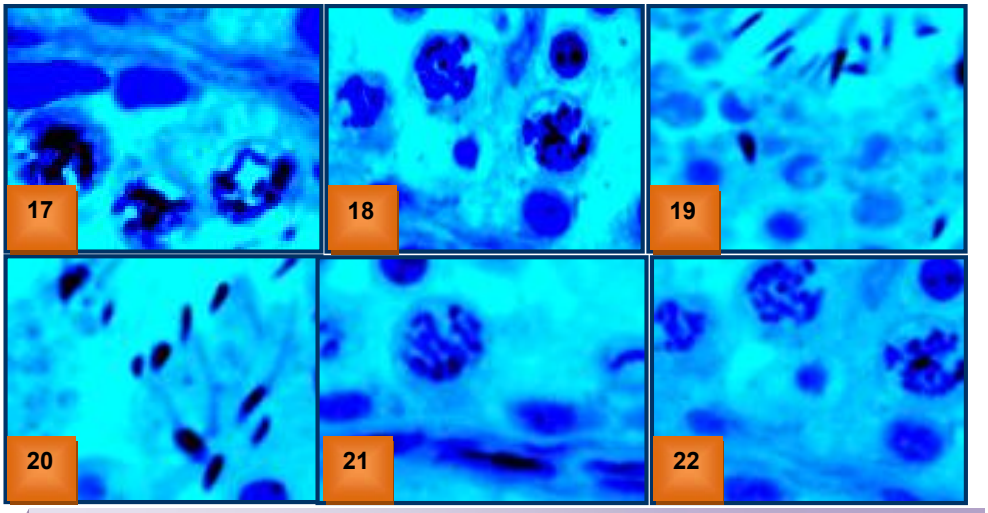

Fig. (17,18): Photomicrograph of mice primary spermatocytes aberrations induced by chloropyrifos at (1/10 LD_{50}) for 90 days. (X 1000)
Fig. (19,20): Photomicrograph of mice primary spermatocytes aberrations induced by chloropyrifos at (1/40 LD_{50}) for 90 days. (X 1000)
Fig. (21,22): Photomicrograph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

4. CONCLUSION:

The results quite indicate that, the percentages of sperm motility decreased in treated mice with each pesticide at the highest concentration. Sperm abnormalities increased in treated mice with all tested pesticides at both concentrations. All the above mention effects were more pronounced with the higher concentration of tested pesticides. Thus, we have to be aware that tested pesticides have detrimental effects on the male reproductive system of rats. Finally, this preliminary investigation gave us clear picture of abnormalities in sperm structure, motility and nuclei morphology, can be caused by tested pesticides, so that we suggest that these pesticides should be used at recommended doses only if necessary.

AUTHORS' CONTRIBUTIONS

Authors may use the following wordings for this section: “H. M. El-bendary 1, designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. ‘S. E. Negm 2, A. A. Saleh 3, M. E. Khadey 4 and F. A. Hosam Eldeen 5 managed the analyses of the study. All authors read and approved the final manuscript.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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