

Lambda, the pyrethroid insecticide as a mutagenic agent in both somatic and germ cells

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Abstract: Cytogenetic evaluations of pyrethroid insecticide cyhalothrin (lambda) were investigated in mice *in vivo* by recording chromosomal aberrations in bone marrow cells and in primary spermatocytes. Cyhalothrin (lambda) insecticide was orally administrated with 2, 2.5, 5 mg/kg b.wt. (1/10, 1/8, 1/4 LD50 doses respectively) for repeated treatment. Cyhalothrin (lambda) was found to produce a significant structural and numerical chromosomal damage after subacute treatment in both bone marrow cells and primary spermatocytes. This effect was dose and time-dependent. For studying sperm abnormalities, mice were orally treated with the highest dose, 1/4 LD50. Cyhalothrin (lambda) insecticide was found to induce a significant increase in the percentage of sperm abnormalities which was mainly in the head. The present study clearly indicates that Cyhalothrin (lambda) insecticide is genotoxic to the different kinds of cells analyzed. [Nature and Science 2010;8(10):72-81]. (ISSN: 1545-0740).

Key words: Pyrethroid insecticides; Lambda-cyhalothrin; chromosomal aberrations; Sperm abnormalities; genotoxicity

1. Introduction

Problems associated with pesticide hazards to man and the environment are not confined only to the developing countries. Developed nations have already suffered these problems, and still facing some problems in certain locations. For many reasons, the severity of pesticide hazard is much pronounced in Third World Countries. The misuse of pesticides by concerned individuals, in addition to lack of or weak national controlling plans is behind the outbreak of adverse effects in developing countries (Mansour, 2004). The indiscriminate use of pesticides and herbicides to increase crop productivity has aroused a great concern among the environmental and health scientists due to their adverse effects in both targets as well as non-target species. Although substantial information is available regarding their environmental and ecological impact, not much is known in regard to its toxicity in the mammalian system (Patel *et al.*, 2007). Pyrethroids (also known as synthetic pyrethroids) are neurotoxic insecticides widely used to control agricultural and domestic insect pests, chemically similar to pyrethrins found in natural pyrethrum extracted from the flowers of chrysanthemum, known for centuries for their insecticidal activity, but are often more toxic to insects, as well as to mammals, and last longer in the

environment than pyrethrins (WHO, 2005, 2003). Apart from their use in agriculture, pyrethroids play an important role in public health programmes. Globally, more than 520 tonnes of active ingredient of pyrethroids is annually used in vector control programmes alone (Zaim&Jambulingam, 2004). The general population is potentially chronically exposed to pyrethroids through food consumption (Fortina *et al.*, 2008). All these compounds are widely distributed in the market and used continuously in our houses. Thus, risks of these pesticides surround us and our sons daily (Kaya *et al.*, 2010). Probabilities of their accumulation in the human bodies are increasingly elevated. Cyhalothrin (lambda), a synthetic pyrethroid type II insecticide, is widely used in Egypt valued for its broad-spectrum control a wide range of pests in variety of applications such as the protection of cotton, cereals, hops, ornamentals and vegetables as well as in public health application against insect, ticks and flies which may act as disease vectors. So, the aim of the present study is to evaluate *in vivo* the genotoxicity of the most commonly used pyrethroid insecticide Cyhalothrin (lambda) in Egypt. The cytogenetic evaluations were conducted on male laboratory mice on three levels: Bone marrow cells as a model of mitotic chromosome abnormalities, Spermatocytes as a model for meiotic

chromosome abnormalities and Sperm count and morphological abnormalities.

2. Materials and Methods.

In this work adult male swiss albino mice (mas muscules) weighing from 25 to 30 gm were used. Cyhalothrin (lambda) 5%EC was chosen to be detected as a broad spectrum synthetic pyrethroid insecticide, used in Egypt. The insecticide under test was administered orally at three different doses 5, 2.5 and 2 mg/kg (bodyweight). To study the effect on both somatic and germ cells, six groups of mice in each case were used each contained six animals. The first and the second groups were treated with the high dose (5mg/kg) for 2 and 4 months respectively. The third and the fourth ones with the median dose (2.5 mg/kg), the fifth and sixth ones with the low dose (2mg/kg), in addition to untreated group which acted as control. Mice were sacrificed 24 hours after the last injection. Each one of these doses was studied as a sub-acute treatment, the dose was given once a week for two different times 2 and 4 months for both somatic and germ studies. Chromosomes were prepared from bone marrow cells to study the abnormalities in somatic cells according to the method of (Yosida and Amona 1965). Chromosomes from germ cells (spermatocytes) were prepared according to (Brewen and Preston 1978) method. Slides were prepared and stained, then so good metaphase spreads for each animal were examined for recording abnormalities in both somatic and germ cells. In the same time sperm morphology was examined for recoding morphological abnormalities using the method of (Wyrobek and Bruce, 1978). Mice were orally administrated with the insecticide (5mg/kg), Cyclophosamide as a positive control (20mg/kg), and a negative control group was used (non-treated) for this experiment. Finally statistical analysis was carried out using analysis of variance (ANOVA) according to (Snedcor and Cochran 1980) and least significant difference (LSD) test according to (Steel and Torrie, 1981).

3. Result Analysis

3.1 Cytogenetic effect on somatic cells

As listed in (table I), the observed types of structural and neumerical abnormalities showed highly significance, even in the low dose (2mg/kg) which showed statistically significant over the control after the two times used 2 and 4 months. In the case of median dose, the significance level was over control and the low dose in most types of structural and numerical aberrations (table1). The

high dose effect showed statistically significance at a level of $P = 0.05$ over the control and the other two treatments for the numerical aberrations (hypoploidy and hyperploids), the increased significance was compared to control and low dose treatment but no significance over the median dose treatment was observed (table1). Also the effect of time was listed in the same table (2 and 4 months) which showed that the increase in the duration of oral treatment increased significantly in all types of structural and numerical aberrations except in the case of centromeric attenuation, which was statistically decreased as the time of duration increased (table1) Also, the same table demonstrated the interaction between doses and times which showed that after the sub acute treatments for 2 months, there was a gradual increase in the number of cells with structural and numerical aberrations as the dose increased which demonstrated high significant increase in the total cells with aberrations as the dose increased (table1). After the sub acute treatment for 4 months, the abnormal cells showed obvious increase as the dose increased.

3.2. Cytogenetic effect on germ cells

The cytogenetic effect of insecticide under test on germ cells was listed in (table2) which illustrated this effect in the following: there was a statistical significance increase in both structural and numerical aberrations over control even with the low dose (2mg/kg) except the cells with polyploidy which showed non- significant comparing to the control (table2). In the case of median dose, the significant level also was over the low dose. In the case of high dose the statistical analysis demonstrated high significance over control and the other two treatments except a chain aberration which showed insignificant when compared with median dose treatment (table2). The effect of time of treatment showed, structural aberrations increased significantly as the time of treatment increased but numerical aberrations showed slight significant decrease as the duration time increased. The genotoxic effect of the insecticide under test showed, gradual increase in number of abnormal germ cells as the duration time of treatment increased, 4 months were more than 2 months. Also, total cells with aberrations showed a dose- dependent increase. i.e increasing the dose increased the total number of abnormal cells significantly over control (Table 2).

3.3. Cytogenetic effect on sperm morphology

(Table 3 and fig1) illustrated the harmful effects of cyhalothrin, the insecticide under test on sperm morphology and sperm count. The results showed that sperm counts were significantly high decreased after treatment with the insecticide than positive and negative controls. The Cyhalothrin (lambda) with highest tested dose (5mg/kg) and Cyclophosphamide (20mg/kg) produced a higher rate of aberrations than the negative control. Cyhalothrin (lambda) under test induced a highly significant increase over the control group in many types of sperm abnormalities which include: amorphous, without hook, banana, small and big for head abnormalities, also for tail abnormalities include coiled tail and two tails, which increased over the control. Cyhalothrin (lambda) also showed a statistically significant increase when compared with Cyclophosphamide treatment in different types of sperm abnormalities. In contrast, it showed statistically non-significant for heads without hook, small head abnormalities and tail abnormalities.

The use of pesticides in food production cannot be avoided especially in developing over populated countries. On the other hand, the application of such chemicals is a major risk due to the danger of being genotoxic and/or carcinogenic and the complete danger is transmitting the mutations to the next generations. Raynauda *et al.* (2008) suggested that carcinogenesis is a multistep process involving multiple mutations and chromosomal aberrations. Also, Au *et al.* (1990) hypothesized that chromosomal aberrations were in the background of carcinogenesis and that the determination of their incidence was an important parameter for the effect of various agents on the health status of mammals and man. Awa (1983) detected a positive correlation between the risk of genetic diseases in populations and the level of cytogenetic damage. The short-term tests for genotoxicity are widely used increasing the potential hazards of such chemicals. In this chapter the results are discussed in an attempt to shed more light on the genotoxicity of Cyhalothrin (lambda) 5% EC (the active substance: -Cyhalothrin, a type II synthetic pyrethroid insecticide), which is widely used in Egypt.

3.4. Effect of insecticide on somatic cell abnormalities

As the results showed, Cyhalothrin (lambda) insecticide caused an increase in many types of chromosomal aberrations. Gaps, breaks, deletions, centric fusions, fragments; hypoploidy, centromeric attenuations, polyploidy and endomitosis were the

main types of aberrations, which were dose-and time-dependent. The observed significant chromosomal aberration after treatment with Cyhalothrin (lambda) insecticide in the present study are in keeping with the results obtained from the experiments performed using formulated lambda-cyhalothrin (Campana, 2003; Celik *et al.*, 2003, 2005, Naravaneni and Jamil, 2005; Georgieva, 2006 and El-Demerdash, 2007). Similar types of aberrations were also detected in mammals under the effects of pyrethroids (Giri *et al.* 2002, Gabbianelli *et al.* 2004, El Khatib *et al.* 2006, Farag *et al.* 2001, 2007 and Quan *et al.* 2010). The presence of gaps and breaks are both indicators of genetic damage as other types of aberrations (Koller, 1973, Anderson & Richardson 1981 and Brogger, 1982 and). Concerning numerical aberrations, according to (Pati and Bhunya 1989) the induction of aneuploidy and polyploidy may be a result of mitotic arrest due to disturbance of spindle which causes a malsegration. Aneuploidies are the most serious and frequent chromosomal defect in humans. Numerical chromosomal abnormalities are associated with congenital defect (Griffin, 1996) and are critical in both the early initiation stages and the progression of a wide array of malignant tumors (parry *et al.*, 2002) Adose-and-time dependent decrease in mitotic index was observed following Cyhalothrin (lambda) insecticide administration. Similar findings were obtained by El-Khatib *et al.* (2006) and Farag *et al.* (2007) who found significant decrease in the mitotic activity after treatment with cypermethrin (a type II synthetic pyrethroid insecticide) and permethrin (a type I synthetic pyrethroid insecticide). The observed dose and time-dependent depression of mitotic activity in the present study may be attributed to the cumulative and cytotoxic effects of Cyhalothrin (lambda) insecticide. Thus in the present study, it has been found that cyhalothrin (lambda) insecticide has the ability to induce chromosomal abnormalities in bone marrow cells which could be an indicator that it may induce chromosomal abnormality in spermatocytes.

3.5. Effect on Germinal cells (meiotic) abnormalities

It is particularly relevant to study the genotoxic effects of pesticides in germinal cells because this is the only system in which transmissible genetic damage from one generation to another takes place (Brewen and prestone, 1978 and William and Hsu, 1980). In the present study, the insecticide under test induced chromosomal abnormality in mice spermatocytes. The abnormalities were in the form of autosomal and X-Y univalent, chain, fragment

aneuploidy and polyploidy. Up to our knowledge, there are not any previous study reports regarding the genotoxicity of Cyhalothrin (lambda) insecticide on spermatocytes of animal. Abnormalities in spermatocytes can result in producing abnormal sperms which can affect the fertility (Ibeh *et al.*, 1994). Alterations in the testis after the insecticide treatment in mice could be due to the direct effect of the substance under test on the testis or indirect effect through the reduction in serum testosterone concentration which is very important for its function (Verma and Nair, 2001). (Muthuviveganandavel *et al.* 2008 and Wang *et al.* 2009), found that cypermethrin one of pyrethroid insecticides used in Egypt increased the malondialdehyde (MDA) content of testis of male rats. Additional to chromosomal abnormalities, a reduction of meiotic division after cyhalothrin (lambda) treatment was also observed. This could be explained by two reasons: the first is the ability of the insecticide to reduce DNA synthesis, which subsequently affect cell division (Sotomayor *et al.*, 2003). The second reason is the toxic effect of the substance under test which affects the rate of cell division (Verma and Nair, 2001). The present study revealed that somatic cell chromosomes were more sensitive to the induced aberrations by cyhalothrin (lambda) pesticide than the germ cell chromosomes. Similar findings were also recorded in mammalian cells (Abd El-Aziz and El Ashmawy, 1989, Abd El-Aziz; El Nahass, 1990, Abd El Aziz *et al.*, 1993; Farag *et al.*, 2001;). Russel (1978) explained that the germ cells are protected from any exposure of chemicals in blood stream by gonadal barriers, which reduce the risk of exposure on germ cells compared to somatic cells.

3.6. Effect of sperm morphology

In the present study cyhalothrin (lambda) insecticide was found to induce abnormalities in sperm shape either in head or tails. The sperm head deformities affect the size of nuclear mass (DNA content) (Wyrobek & Bruce, 1978 and Wyrobek *et al.*, 1984) whereas the tail deformity gives an impression of limitation of sperm movement which causes reduction of fertility in human and experimental animals (Lancranijan *et al.*, 1975). A statistically significant increase in the number of abnormal sperms occurred after treatment with the high dose (5mg/kg) of cyhalothrin (lambda) insecticide compared with the control. This increase was mainly in the head rather than in the tail. The reduction in sperm count and motility after the insecticide

cyhalothrin (lambda) treatment means, increase incidence of sperm abnormalities and subsequently reduction in fertility according to the regardation of (Agnes and Akborsha, 2003). Previous studies revealed that exposure to pesticides has been associated with reproductive dysfunction, such as spontaneous abortions, infant prematurity, congenital malformations, reduced fertility, sperm mortality decrease and hyperploidy/polyploidy in the spermatozoa of exposed men (Oliva *et al.*, 2001, Recio *et al.*, 2001, Crisototomo and Molina, 2002;; Chauhan and Gupta 2005, qua *et al.*, 2008 and Nada *et al.*, 2010). The positive correlation between cytogenetic damage and sperm abnormality in the present study was also reported by (Bernardini *et al.*, 1998 and Xia *et al.*, 2004).

4. Conclusions

In this study, cyhalothrin (lambda) insecticide was found to be genotoxic on both somatic and germinal cells; which was dose and time dependent, also the statistical analysis revealed that cyhalothrin (lambda) insecticide is more genotoxic on bone marrow cells than germinal cells. It was shown that cyhalothrin (lambda) insecticide has a damaging effect on the sperm morphology, which may be the cause for infertility and abnormal embryos. Thus care must be taken when handling this insecticide. Educating people about pesticide safety is important and encourage them to buy fresh and processed organic foods thorough system of administration has been put in place to control the advertisement, sale, storage, supply and use of pesticides.

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Table(1): Effect of dose, time and their interaction on bone marrow structural and numerical aberrations.

Treatments	Structuralaberation (M±Sd)										Numericalaberation (M±Sd)					Total Abenations(M±Sd)
	Chromated Gap	Chromos. Gap	Chromated Break	Chromos. Break	Centric fision	End to end	Fragment	Total	Centrom. atune.	Hypoploid	hyper ploid	Polyploidy	Endomitis	Total		
Control	5.83b ±0.55	0.50c±0.21	1.33 c±0.64	0.33c ± 0.01	1.83 d ±0.94	2.17 d ± 0.55	0.83 d ± 0.19	0.00 c ± 0.00	12.83 d ± 3.18	4.50 c± 0.57	2.67 c ± 0.39	0.00 d ± 0.00	0.00 b ± 0.00	3.00 b ±0.99	10.17 d± 1.89	23.00 d ± 4.27
1/10 LD ₅₀	17.33a ±4.14	3.17b±2.10	3.17b±0.19	0.50 bc ± 0.18	4.00 c± 1.48	4.67 b ± 0.78	5.00 c ± 2.05	0.33 c ±0.37	8.17 c± 11.67	10.17 b± 0.77	6.67 b ± 0.78	0.33 c ± 0.07	0.50 b	0.83 c ± 0.19	18.50 c ± 1.99	56.67 c ± 10.73
1/8 LD ₅₀	18.83a ±3.60	7.00a±2.83	6.17a±2.91	0.67 b ± 0.83	6.17 b ± 1.66	4.00 c ± 0.39	6.50 b± 1.75	1.50 b ± 0.97	50.83 b ±13.53	10.33 b± 1.22	8.67 a ± 0.59	1.83 a ±1.28	1.33 a	4.83 b ± 3.45	27.00 b ± 4.84	77.84 b ± 17.88
1/4 LD ₅₀	18.83a ±3.60	7.17a±3.08	5.67a±1.60	2.17 a ± 1.28	9.34 a ± 0.84	5.00 a ± 0.14	7.83 a ± 2.54	4.17 a ± 1.18	60.17 a ± 11.36	13.43 a ± 0.87	8.83 a ± 2.10	0.67 b ± 0.73	0.50 b± 0.55	8.83 a ± 2.47	32.17 a ± 2.24	92.33a±12.46
LSDat 0.05	1.96	1.35	1.23	0.28	0.39	0.24	0.99	0.56	5.85	0.63	0.57	0.07	0.71	2.05	2.61	6.21
2 m	13.25b ±5.22	2.75b±2.21	3.17b ± 1.59	0.42b ±0.38	4.25b ±2.99	3.75b ± 1.32	3.67b± 2.11	1.00b ± 1.45	32.25b± 16.27	10.00a± 3.86	6.42b±2.59	0.58b ±0.52	0.25b	3.42b ± 3.3	20.67b ± 8.75	52.92b±24.65
4. m	17.17a ± 6.72	6.17a±3.96	5.00a±3.01	1.42a ±1.25	6.42a± 2.86	4.17a± 1.13	6.42a± 3.58	2.00a ± 2.08	48.75a± 22.02	9.17b±2.89	7.00a±3.01	0.83a± 1.32	0.92a±	5.33a ± 3.86	23.25a ± 9.45	72.00a ± 30.88
LSDat 0.05	1.39	0.95	0.873	0.197	0.27	0.167	0.69	0.39	4.14	0.45	0.40	0.05	0.50	1.45	1.84	4.39
Control	5.33e ± 0.04	0.33c±0.17	1.00f ± 0.10	0.33de± 0.02	1.00g ± 0.01	1.67e±0.01	0.67f± 0.04	0.00d ±0.00	10.33e ±2.34	4.00f±0.26 3.0260	2.33e±0.17	0.00e± 0.00	0.00b±0.00	2.67b ± 1.04	9.00d±1.67	19.330 ± 1.04
1/10 LD ₅₀	13.67d ± 1.09	1.33c±0.64	3.00de	0.33de± 0.02	2.67f±	4.00c±0.26	3.33e± 1.04	0.00d ± 0.00	28.33d± 4.81	10.67c± 0.66	7.33c±0.33	0.33d± 0.11	0.00b	0.67b ± 0.04	19.00c ± 2.76	47.33d±2.43
1/8 LD ₅₀	16.00cd± 1.98	4.67b ± 1.04	3.67cd	0.00e	4.67e± 0.28	4.33b±0.21	5.00d ± 0.54	0.67d ± 0.04	39.00c± 5.48	11.33c±0.43	9.00b±0.34	0.67c± 0.04	0.00b	2.00b ± 0.50	23.00b ± 2.29	62.00c ± 6.32
1/4 LD ₅₀	18.00bc± 2.28	4.67b±1.64	5.00be	1.00bc± 0.10	8.67 b±	5.00a±0.20	5.67cc± 0.82	3.33b ± 0.43	51.33b± 6.29	14.00a± 0.35	7.00c±0.29	1.33b± 0.07	1.00b	8.33a ± 2.34	31.67a ± 2.66	83.00b ± 8.65
1/10 LD ₅₀	21.00a ± 1.10	5.00b ± 0.74	3.33cde±0.01	0.67cd± 0.04	5.33d±0.33	5.33a± 0.33	6.67b ± 1.07	0.67d ± 0.04	48.00 b±5.22	9.67d±0.55	6.00d±0.26	0.33d ±0.02	1.00b	1.00b ± 0.10	18.00c ± 1.26	66.00c ± 4.56
1/8 LD ₅₀	21.67a ± 2.09	9.33a ± 1.64	8.67a ±	1.33b±0.64	7.67c± 0.15	3.67c±0.16	8.00b ± 0.79	2.33c ± 0.51	62.67a ± 2.74	9.33d±0.74	8.33b±0.66	3.00a ± 0.10	2.67a	7.67a ± 2.34	31.00a ± 2.29	93.67a ± 2.66
1/4 LD ₅₀	19.7ab±2.15	9.67a ± 1.51	6.33b ± 1.07	3.33a	10.00a± 0.65	5.00a±0.10	10.00a± 1.18	5.00a ± 1.10	69.00a ± 7.01	12.67b ± 0.66	10.67a±0.94	0.00e± 0.00	0.00b	9.33a±3.01	32.67a ± 2.17	101.67a±7.21
LSDat 0.05	2.78	1.90	1.75	0.395	0.545	0.33	1.39	0.79	8.28	0.89	0.80	0.095	1.01	2.89	3.69	8.78

Statistical analysis of results were done according to least significant difference (LSD) test Different letters (a , b, c, d, e) within each column means the degree or significance at 0.05 level. (M ±Sd): M is the mean value, Sd: Standard deviation.

Table (2): Effect of dose, time and their interaction on structural aberration and numerical aberrations of Spermatoocytes

Treatments	Structural aberration (M ± Sd)						Numerical aberration (M ± Sd)				Total aberrations (M ± Sd)
	Autosomal univalent	X-Y univalent	Chain	Fragment	Ring	Total	Hypoploidy	Hyperploidy	Polyploidy	Total	
Control	3.167 d± 0.93	2.334 c± 1.01	0.000 ± 0.00	0.000 d± 0.00	0.000 d± 0.00	5.500 d± 1.44	2.833 d± 0.55	0.000 c± 0.00	2.500 c± 0.55	5.334 d± 1.00	10.834 d± 1.32
1/10 LD ₅₀	9.833 c± 1.89	9.167 b±2.56	3.333 b± 0.03	0.334 c± 0.37	0.334 c± 0.37	23.000 c± 5.28	8.000 c± 0.67	0.000 c± 0.00	1.667 c± 0.76	8.833 c± 1.28	31.833 c± 4.76
1/8 LD ₅₀	15.000 b± 3.07	10.000 b ± 1.44	4.334 a± 0.40	0.833 b± 0.55	0.500 b± 0.21	30.667 b± 5.02	10.333 b± 0.97	0.834 b ± 0.19	4.167 b± 2.52	15.333 b± 2.88	46.000 b± 2.81
1/4 LD ₅₀	17.167 a± 1.65	11.834a± 2.56	4.500 a± 0.37	2.000 a± 1.14	0.834 a± 0.18	36.333 a± 5.43	12.334 a± 1.73	2.167 a± 0.55	6.334 a± 2.32	20.833 a± 3.17	57.167 a± 2.89
LSD at a 0.05	1.31	1.29	0.25	0.21	0.08	2.47	1.02	0.04	1.20	1.99	1.79
2 M 4 M	9.917 b± 6.37	6.917 b± 3.27	2.917 b± 1.82	0.333 b± 0.44	0.334 b± 0.35	20.417 b± 10.53	8.583± 4.38	0.583 b± 0.71	4.500 a± 3.07	13.667 a± 7.62	34.083 b± 17.76
4 M	12.667 a±5.13	9.750 a± 4.53	3.167 a± 1.99	1.250 a± 1.18	0.500 a± 0.40	27.333 a± 13.99	8.167± 3.21	0.917 a± 1.14	2.833 b± 1.31	11.500 b± 5.14	38.833 a± 18.55
LSD at a 0.05	0.93	0.92	0.18	0.15	0.06	1.75	N.S	0.03	0.85	1.408	1.26
Control x2 M	3.000 e± 1.02	2.000 e± 1.08	0.000 d± 0.00	0.000 f± 0.00	0.000 d± 0.00	5.000 e± 1.10	2.333 d± 0.02	0.000 e± 0.00	2.667 c± 0.03	5.000 e± 1.03	10.000 g± 1.08
Control x4 M	3.333 e± 1.03	2.667 e ± 1.03	0.000 d± 0.00	0.000 f± 0.00	0.000 d± 0.00	6.000 e± 1.79	3.333 d± 0.03	0.000 e± 0.00	2.333 c± 0.82	5.667 e± 1.06	11.667 g± 1.06
1/10 LD ₅₀ x2 M	8.333 d± 1.06	7.000 d± 1.07	3.333 c± 0.03	0.000 f± 0.00	0.000 d± 0.00	18.667 d± 1.63	8.000 c± 1.03	0.000 e± 0.00	1.000 c± 0.10	9.000 d± 1.67	27.667 f± 1.66
1/10 LD ₅₀ x4 M	11.333 c± 1.03	11.333 b± 1.06	3.333 c± 0.03	0.667 d± 0.03	0.667 b± 0.03	27.333 c± 3.27	8.000 c± 0.26	0.000 e± 0.00	2.333 c± 0.34	8.667 d± 1.09	36.000 e± 1.35
1/8 1.1)50x2 M	12.333 c± 1.06	9.000 c± 1.05	4.000 b± 0.27	0.333 e± 0.04	0.667 b± 0.03	26.333 c± 1.51	10.333 b± 1.03	0.667 d± 0.03	6.333 ab± 1.34	17.333 b± 2.42	43.667 d± 1.44
1/8 1.1)50 x4 M	17.667 ab± 1.06	11.000 b± 1.03	4.667 a± 0.03	1.333 b± 0.03	0.333 c± 0.17	35.000 b± 2.10	10.333 b± 1.12	1.000 c± 0.07	2.000 c± 0.10	13.333 c± 1.60	48.333 c± 1.16
1/4 LD ₅₀ x2 M	16.000 b± 1.27	9.667 bc± 1.06	4.333 ab± 0.51	1.000 c ± 0.21	0.667 b± 0.03	31.667 b± 0.82	13.667 a± 1.03	1.667 b± 0.03	8.000 a± 1.83	23.333 a± 2.01	55.000 b± 1.74
1/4 1.1)50 x4 M	18.333 a± 1.06	14.000 a± 1.09	4.667 a± 0.03	3.000 a± 0.44	1.000 a± 0.03	41.000 a± 2.76	11.000 b± 1.03	2.667 a± 0.03	4.667 b± 1.33	18.333 b± 1.51	59.333 a± 1.93
LSD at a 0.05	1.86	1.84	0.35	0.30	0.11	3.49	1.45	0.06	1.69	2.815	2.52

Statistical analysis of results were done according to least significant difference (LSD) test Different letters (a , b, c, d, e) within each column means the degree or significance at 0.05 level. (M ± Sd): M is the mean value, Sd: Standard deviation.

Table.3. Effects of control + ve, control –ve and 1/4 LD50 on sperm morphology

Treatments	Head Abnormalities (M ± Sd)							Tail Abnormalities (M ± Sd)			Total aberrations (M ± Sd)	Total count of sperm ×10 ⁶ (M ± Sd)
	Normal	Without hook	Big head	Small head	Banana	Amorphous	Total head	Two tail	Coiled tail	Total tail		
-VE	95.4a ± 0.86	0.96c ± 0.26	0.48c ± 0.30	1.28b ± 0.50	0.68c ± 0.41	1.16b ± 0.26	4.6c ± 0.864	0.00b ± 0.00	0.00b ± 0.00	0.00b ± 0.00	4.56c ± 0.86	5.93a ± 0.31
1/4LD50	73.7c	3.52b	4.12a ± 1.24	4.96a ± 1.77	4.36a ± 0.98	8.56a ± 4.89	25.5a ± 3.651	0.44a ± 0.30	0.32a ± 0.46	0.76a ± 0.52	26.28a ± 3.85	2.11c ± 0.31
+VE	78.9b ± 1.78	6.08a ± 0.39	2.08b ± 0.23	5.24a ± 0.61	2.48b ± 1.11	4.48b ± 0.30	20.4b ± 1.705	0.56a ± 0.22	0.20a ± 0.14	0.76a ± 0.22	21.12b ± 1.78	3.55b ± 0.33
LSD at a 0.05	3.443	0.800	1.030	1.544	1.226	3.903	3.279	0.167	0.292	0.447	3.443	0.427

A b c d e
f g h

Fig. (1): Sperm morphology of treated mouse.

- (a) Normal sperm morphology of control mouse.
- (b) Sperm of treated mouse showing head without hook.
- (c) Sperm of treated mouse showing big head.
- (d) Sperm of treated mouse showing small head.
- (e) Sperm of treated mouse showing banana shaped head.
- (f) Sperm of treated mouse showing amorphous head.
- (g) Sperm of treated mouse showing two tails.
- (h) Sperm of treated mouse showing coiled tail.

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