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RESEARCH ARTICLE

Effects of the Pesticide Cyren on Chromosomes and Sperms of Albino Males Mice

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ABSTRACT

Cyren (Chlorpyrifos 500 g +Cypermethrin50g/Liter) is one of the widely used organophosphate insecticides. The aim of this study was to evaluate the genotoxicity of this insecticide in males of Swiss albino mice *Mus musculus* BALB/c strain. The tested parameters were chromosomal aberrations (CA) and sperm abnormalities to evaluate possible damage effects on genetic material and sperms. The pesticide was administered orally to male mice in four different doses (0, 10, 20 and 30 mg/kg of body weight). The mice were killed after three or six weeks of treatment. The results of chromosome aberration assay revealed that all the tested doses and periods induced chromosomal aberrations (CA) such as centromeric gaps, chromatid gaps, chromatid deletion, dicentric chromosome, and ring chromosome. The results of sperm abnormality assay revealed that Cyren has the ability to induce sperm abnormalities in all doses used compared to untreated mice, which represented by hookless sperm, swollen head sperm, amorphous head sperm, defective hook sperm, banana head sperm, double head sperm, double tail sperm, bent midpiece defect and coiled tail.

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INTRODUCTION

Although there are benefits to the use of pesticides in agriculture, there are also drawbacks, such as potential toxicity to human and animals (Soloneski and Larramendy, 2012; Sanborn, 2004). According to the Stockholm Convention on Persistent Organic Pollutants, 10 of the 12 most dangerous and persistent organic chemicals are pesticides (Gilden*et al*., 2010). Fortunately, a large portion of the chemicals introduced to the environment are gradually broken down and assimilated by natural process, nevertheless studies had shown that the concentration of toxic residues of the pesticides rises gradually through the food chain about ten times, because living organism cannot get rid of these toxin and store them in different tissues (Mohn, 1973).

Most of pesticide had been tested for their genotoxicity and cytogenicity using different testing assays (Soloneski and Larramendy, 2012). The results showed different types of chromosomal aberrations and gene mutations, for example Bhunya and Behera (1987) reported that Tri-chloro acetic acid induced chromatid aberrations in mouse *in vivo* test system and the effects were time, dose, and route dependent. Other experimental studies proved that chlorpyrifos induced genotoxicity (Rahman*et al*., 2002; Ali *et al*., 2009) and sister-chromatid exchanges (Amer&Aly, 1992; Sobti*et al*., 1982), while Degraeve and Moatschen (1984) reported that organophosphorus insecticide Malathion does not cause much increase in the percentage of chromosomal aberrations. Five pesticides were studied for their mutagenicity in bone marrow cells of Swiss mice, culture of human peripheral lymphocytes and the yeast's *Saccharomyces cerevisiae*. The results revealed that Dimatyph (diaethyleneimidamido-thio-phosphorous acid) induced potent mutagenic effect in all three test–systems, Endosulfan was genetically active in mice and yeasts, Cypermethrin increased the level of chromosomal aberrations in mice bone marrow cells only at a high dose, Picloram was genetically active only in yeasts and Crotoxophos did not reveal any mutagenicity in all tests used (L`vova, 1984). Rupa *et al.* (1991) studied the cytogenetic effect of Quinalphos in Swiss albino mice using the micronucleus test, bone marrow and germ cell chromosome assays. The results demonstrated that Quinalphos induced the formation of micronuclei and caused a significant increase in chromosomal aberrations in the bone marrow cells at 10 and 15 mg/kg body weight and in germ cells at all tested doses.

Using chromosome aberration (CA), bone marrow micronucleus (MN) and sperm abnormality assays in mice the genotoxic effects of carbosulfan were evaluated by

Giri *et al.* in 2002. All the doses of carbosulfan induced significant dose-dependent increase in the frequency of chromosome aberration, micronucleated polychromatic erythrocytes (PCEs) and sperm head abnormalities but did not affect the total sperm count

Effects of Pesticides on Sperm

The survival of a species depends on the integrity of its reproductive system. Damage by physical or chemical agents to the sperm, ovum or fertilized ovum may cause infertility, spontaneous abortion and birth defects, or may result in mutations that are passed on to future generations (Swati, 2004). Ware and Good (1967) revealed that male and female rats treated with Mirex, Telodin and DDT (dichlorodiphenyltrichloroethane) suffered from reproductive impairment of various types. Many other pesticides, Aldrin, Dieldrin and Chlordane not only cause-toxicity, carcinogenicity, teratogenicity but also adversely affected the reproduction system (Deichmann *et al*., 1971). Cytogenetic evaluations of pyrethroid insecticide cyhalothrin (lambda) by Abdel-Aziz and Abdel-Rahem (2012) in mice *in vivo* revealed a significant structural and numerical chromosomal damage after sub acute treatment in both bone marrow cells and primary spermatocytes. The pesticides Raxil and Granstar have the ability to cause chromatid and chromosomal aberrations in male of treated mice, also both pesticides have the ability to produce abnormalities in the sperms of the exposed male mice, by reducing the productivity, fertility and increasing sterility of exposed mice, in addition both pesticides have the ability to inherit sperm abnormalities to the first generation (Taha, 2000). Chlorpyrifos at dose levels of 7.5, 12.5 and 17.5 mg/kg of body weight /day was administered orally to male rats for 30 days to evaluate the toxic alterations in testicular histology, biochemistry, sperm dynamics and testosterone levels, the results concluded that Chlorpyrifos induces severe testicular damage and results in the reduction in sperm count and thus affect fertility (Joshi *et al.,* 2007). The literature offers many other examples showing the effects of pesticides on the genetic materials and sperms of different mammalians.

Cyren is one of organophosphate pesticide that is widely used for crops protection. Product safety sheet data of Cyren indicate that this insecticide is highly toxic to birds and reptiles and very highly toxic to fish and aquatic invertebrates. The aim of this research was to investigate the effects of Cyren on chromosomes and sperms of mice, because little information is available on its toxicity.

MATERIALS AND METHODS

Reagents

Cyren, in the form of Chlorpyrifos 500 g + Cypermethrin 50g/Liter was received from General Directorate of Agriculture in Dohuk city. The LD_{50} of Cyrenin albino mice was found to be 60 mg/kg bw. (Cyren Safety Data Sheet, 2010). Cyren was used in three doses 10, 20, and 30 mg/kg of body weight. The doses were prepared by diluting the insecticide with distilled water then placed in a clean and dry bottle. The mice were treated orally 3 times weekly using dosage syringe prepared locally from 2 ml disposable syringe and needle.

Animals

Adult Swiss albino mice (*Mus musculus*) BALB/c 8-10 weeks in age, weighing 30-35 gm were used for breeding in this study. All aspects of the animal experiment, breeding, parturitions were carried out in the Animal House of the Department of Biology, Faculty of science, University of Zakho and maintained at room temperature 22 ± 2 ^{0}C. A standard diet and water were used to feed the mice. Five mice were used for each treatment with total of 40 mice in the experiment.

Experimental Design

The experiment was carried out in a Factorial Design arranged in a Completely Randomized Design (CRD). The main factors were: **A-Cyren doses**. The doses were 0.0, 10, 20, 30, mg/kg b.w. and **B-Periods** 3weeks and 6 weeks.

Preparation of Chromosome from the Bone Marrow Cells

At the end of the treatment, each animal was injected intraperitoneally with 1ml of fresh colchicine (0.04 %) to arrest cell division at metaphase. Two hours after injection, animals were sacrificed by cervical dislocation for preparation of the chromosomes from bone marrow cells. Chromosomes were prepared by using the methodology of Evans *et al*. (1964). Both femurs were dissected out and cleaned from any adhering muscle tissue. Bone-marrow cells were collected from both the femurs by flushing in saline solution and then incubated at 37°C in hypotonic solution (KCl 0.56 %) for 35 min, fixed in methanol-glacial acetic acid (3:1). The cells were re suspended in a small volume of fixative. Using Pasteur pipette, 3-5 drops of cells suspension were dropped from appropriate height (3 feet) on a clean moist slide, and then the slides were air dried at room temperature, stained with 2 % Giemsa′s stain for 10-15 min then washed with phosphate buffer to remove the excess stain. At least 100 metaphase cells per animal were scored to investigate chromosomal aberrations (Sharma and Sharma, 1980).

Sperm Preparation

The sperms were prepared from epididymis and vas deferens. After killing the animals, the epididymis and vas deferens were removed from the reproductive system andtransferred to a small petri dish containing normal saline. Using a sharp scissor the epididymis and vas deferens were cut into several parts, and the sperms were released into saline solution.

The sperm suspension was smeared, dried, fixed with fixative (three volumes of absolute methanol and one volume of glacial acetic acid.), then stained with haematoxylin for 15 min, washed with tap water, after that stained with 1 % eosin for 10 min and washed with tap water and left to dry at room temperature. At least 1000 sperms were counted from each animal to determine sperm morphology and abnormalities (Wyrobek, 1979).

Statistical Analysis

Statistical analyses were performed with SAS software. Data were analyzed using one way analysis of variance (ANOVA) followed by Duncan's multiple range test used to test the significant differences between means

of the doses and periods (Duncan, 1955). Results are reported as mean values ± S.E. and differences were considered as significant at (P≤ 0.05).

RESULTS

Effects of Cyren on chromosomes of bone marrow cells of Mice

The frequencies of different types of structural chromosomal aberrations (CA) induced by Cyren in bone marrow cells were shown in Table 1. The resulted show asignificant differences ($p<0.01$) in chromosomal aberrations such as centromeric gaps, chromatid deletion, chromatid gaps, dicentric chromosome and ring chromosome when compared to untreated mice. These types of aberrations were shown in Figure 1. The data in Table 1 also reveal great differences in the total number of aberrations. The values increased with increase Cyren doses as well as with extending treatments period.

The results in Table 1 indicate that the most types of aberrations were the centromeric gaps and ring chromosomes at D_3 (11.10±0.64 and 9.70±0.47,

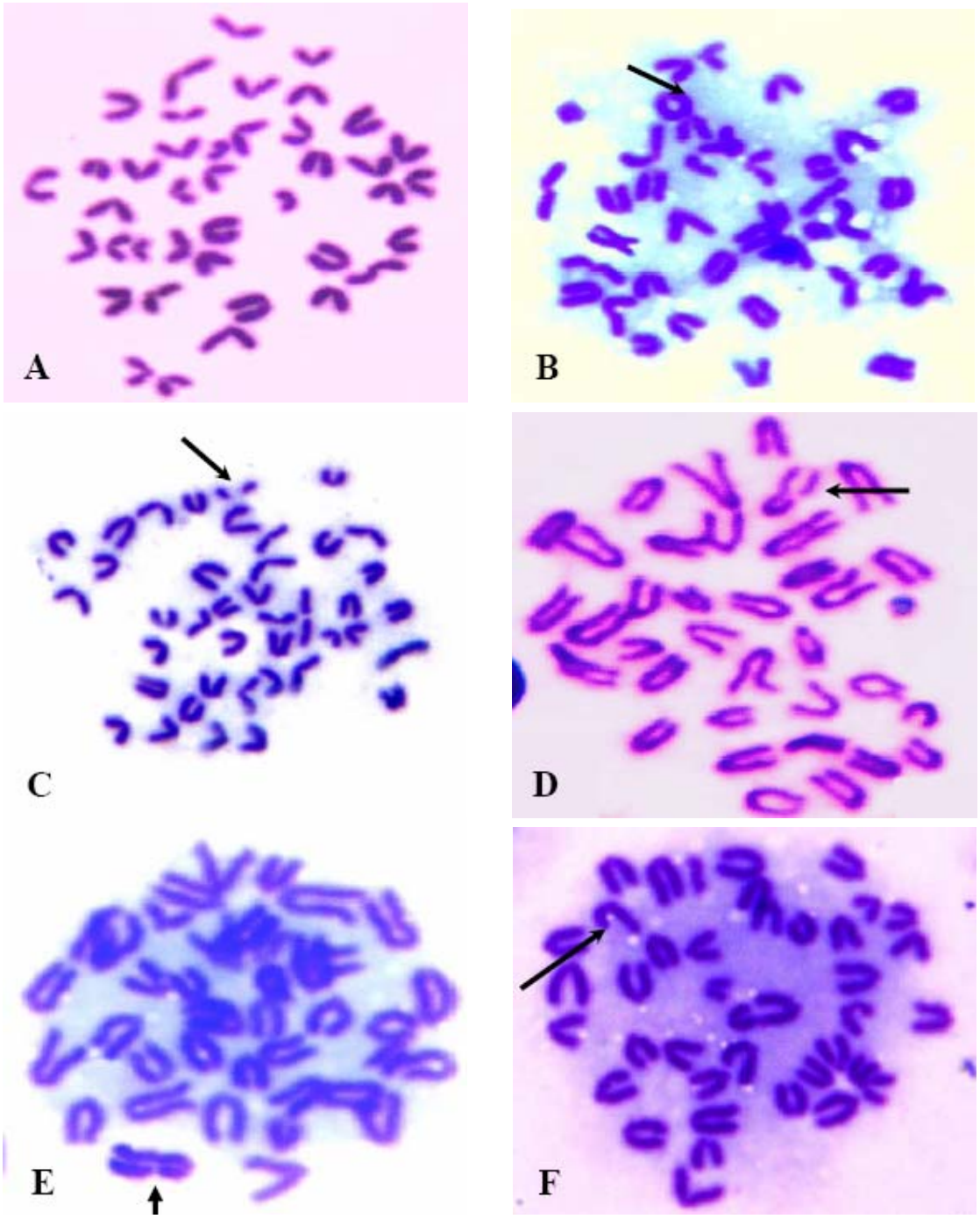

Fig. 1: Different types of Chromosomal Aberrations Induced by Cyren Insecticide in Males Albino Mice (1000X). A: Normalchromosomes, B: Ring chromosome, C: Centromeric gap, D and E: Chromatid gap, F: deletion.

The results in Table 1 indicate that the most types of aberrations were the centromeric gaps and ring chromosomes at D_3 (11.10±0.64 and 9.70±0.47, respectively) when compared to control D_0 (2.50±0.42 and 2.10±0.23, respectively). The least CA was in the dicentric chromosomes, control treatment was 0.30±0.15 increased to 2.00±0.36 in D_3.

The number of CA increased with extending the doses period as shown in Table 1. There was a significant effects ($p<0.01$) of periods on centromeric gaps, chromatid gaps and ring chromosomes and significant effects ($p<0.05$) on chromatid deletion and no effects on dicentric chromosomes.

Significant interactions ($p<0.05$) between doses and periods has been found in centromeric gaps and ring chromosomes while interaction in the rest of the parameters were none significant (Table 1).

The highest interaction value scored in D_3P_2 12.80±0.37 for centromeric gaps and the least value scored in D_1P_1 1.20±0.37 for dicentric chromosomes.

Effects of Cyren on Sperms of Males Albino Mice

The data in Tables 2 A and B represent the effects of Cyren on the sperms of Males Albino Mice. The values of these traits in Tables 2 A and B were the number of abnormal sperms counted from total of 1000 sperms. From these data, it was obvious that there were high significant difference in all types of sperm abnormalities listed in the table due to the effects of the treatments when compared to control. The total number of abnormal sperms increased constantly with the increase of Cyren doses. The highest total number of abnormal sperms was in D_3 = 148.7. The highest mean value of abnormal head sperms in treated animals (Table 2.A) compared to other types of head abnormalities was 31.90±3.11 in amorphous head sperm. The highest mean value of abnormal sperms tail (Table 2.B) compared to other types of tail abnormalities was 36.80±2.81 in bent mid piece defect. The least affected trait was the double tail sperms with value of 0.10±0.10b in D_0 treatment. There were a high significant differences ($p<0.01$) between all doses due to the effects of periods as the total number of affected sperm in the first period was 136.75 increased to 214.20 in the second period. The most affected traits by the periods were sperms with bent mid piece defect and sperms with coiled tail, while the least affected trait was the sperms with double tail trait (Table 2 B).

There was a significant differences ($p<0.01$) between treatments due to the interactions between doses and periods (Tables 2 A and B) the only non-affected trait was sperms with double tail. The highest value of total abnormal sperm was in D_3P_2 with value of 186.2, while the least interaction was in D_0P_1 with total abnormal sperms 38. The most interaction between the periods and doses was 44.00±2.81 occurred in sperms with bent mid piece defect, while the least value of interactions was 0.00±0.00 in D_0P_1 for sperms with double head.

Most types of sperms abnormalities in males albino mice induced by Cyren insecticide are shown in Figure 2.

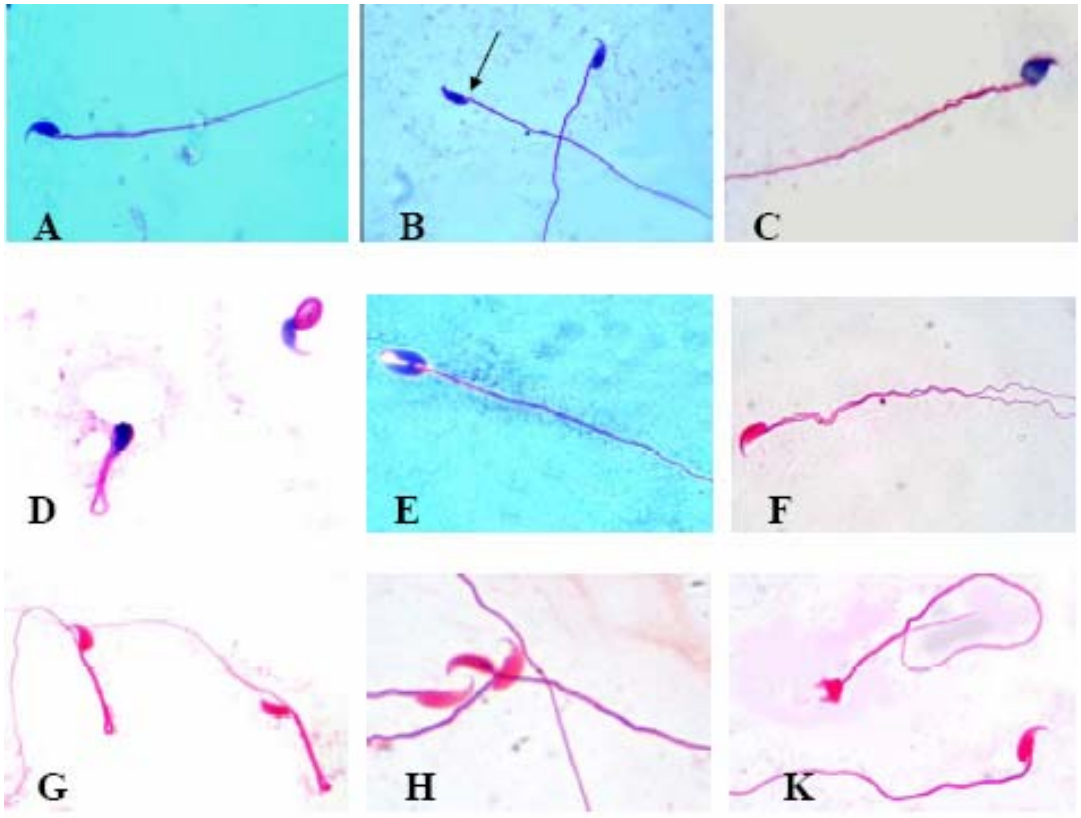

Fig. 2: Shows Most Kinds of Sperm Abnormalities Induced by Cyren Insecticide in Swiss Albino Mice (1000X). A: Normal sperm; B: Hookless sperm, C: Swollen head sperm, D: Coiled tail defect, E: Double head sperm, F: Double tail sperm, G: Midpiece bent defect, H: Banana head sperm, K: Amorphous head sperm.

Table 1: Mean ± S.E for the Effects of Cyren (Doses, Periods and their Interactions) on Chromosome Aberrations in Bone Marrow Cells of Males Albino Mice

Factors		Chromosome Aberrations					
		Centromeric gaps	Chromatid deletion	Chromatid gaps	Dicentric chromosome	Ring chromosome	Total-percent aberrations
Doses	D_0(control)	2.50±0.42c	0.20±0.13c	1.10±0.10c	0.30± 0.15b	2.10±0.23c	
	D_1	8.80±0.78b	1.70±0.26b	4.90±0.43b	1.30±0.21ab	8.00±0.59b	
	D_2	10.00±0.73ab	2.30± 0.30ab	5.70±0.47ab	1.60±0.22a	8.80±0.35ab	
	D_3	11.10± 0.64a	2.80±0.35a	6.30±0.49a	2.00± 0.36a	9.70±0.47a	
	Significant	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	
Perio	P_1	6.75±0.67b	1.45±0.24b	3.75±0.42b	1.20±0.21	6.35±0.61b	
	P_2	9.45±0.96a	2.05±0.32a	5.25±0.58a	1.40±0.23	7.95±0.80a	
	Significant	p<0.01	p<0.05	p<0.01	N.S	p<0.01	
Interactions(Doses and Periods)	D_0P_1	2.40±0.60f	0.20± 0.20	1.00±0.00	0.40±0.24	2.00±0.31d	
	D_0P_2	2.60±0.67f	0.20±0.20	1.20±0.20	0.20±0.20	2.20±0.37d	
	D_1P_1	6.80±0.66e	1.40±0.24	4.20±0.58	1.20±0.37	6.60±0.24c	
	D_1P_2	10.80±0.58bc	2.00±0.44	5.60±0.50	1.40±0.24	9.40±0.74b	
	D_2P_1	8.40±0.67de	1.80±0.37	4.60±0.50	1.40±0.24	8.20±0.48b	
	D_2P_2	11.60±0.81ab	2.80±0.37	6.80±0.37	1.80±0.37	9.40±0.40b	
	D_3P_1	9.40±0.50cd	2.40±0.50	5.20±0.48	1.80±0.58	8.60±0.50b	
	D_3P_2	12.80±0.37a	3.20± 0.48	7.40±0.50	2.20±0.48	10.80±0.37a	
	Significant	p<0.05	N.S	N.S	N.S	p<0.05	

Note: Similar letters in each column refer to non significant difference while different letters refer to significant difference between them; N. S= Non significant

DISCUSSION

Effects of Cyren on chromosome aberrations in bone marrow cells of mice

The experimental results showed significant differences between doses in their effects on all types of chromosomal aberrations. The high doses led to higher ratio of aberrations. Similar results were obtained by Giri*et al*. (2002) and Abdel-Aziz and Abdel-Rahem (2010) who observed that increasing dose of pyrethroid insecticide significantly increased the chromosomal aberrations and sister chromatid exchanges in bone marrow cells of Swiss albino mice. Moreover, Amer and Aly (1992) also observed similar results with the insecticides Gardon and Dursban which induced a high percentage of metaphases chromosomal aberrations in cultured mouse spleen after four hours of treatment,also the frequency of sister chromatid exchange increased with increasing concentration of the insecticides. Balaji and Sasikala (1993) also noticed that Malathion show a dose-dependent increase in the frequency of chromosomal aberrations, as well sister chromatid exchanges in human peripheral leukocytes. Similarly, Sierra *et al*. (1998) also observed that Miral (an organophosphorus insecticide) is capable of inducing chromosomal aberrations at high doses in mice. It was clear that the frequency of chromosomal aberrations increased as the dose of insecticide increased indicating dose-dependent increase in chromosomal aberration induced by Cyren. The highest concentration of Cyren was most effective to cause all types of chromosomal aberrations; this may be due to the elevation of cells sensitivity to higher concentration because high dose of mutagens causes a rapid accumulation of mutated genes within the cells.

The results also revealed significant differences between periods in their effects on the increase of chromosomal aberrations except (dicentric chromosome). Al-Attar (2004) observed similar results when he used the insecticides Lorsban. These results also agree with results reported by Yadav and Chhillar (2001) who certified that the frequency of chromosomal aberrations in human blood lymphocytes showed elevation with prolonging the duration of exposure to roadsides pollutants, as well Stocco*et al*. (1981) explained that chromosomal aberrations increased with increasing exposure time to organo phosphorus pesticides in cultured lymphocyte cells of cattle (*Bostaurus Taurus*).

Effects of Cyren on Sperm Abnormalities of Albino Males Mice

In the present study Cyren insecticide was found to induce abnormalities in sperm shape either in head or tails (Table no. 2A&B).A statistically significant increase in the number of abnormal sperms occurred after treatment with Cyren especially with the third dose (30 mg/kg) compared to control.These results are similar to those reported by Bhunya and Behera, (1988) who observed that the insecticide Monocrotophosincreased sperm head abnormalities rate with increasing the dose. The results as well are similar to those reported by Al-Attar (2004) and Al-Assady (1994). A positive correlation between cytogenetic damage and sperm abnormality also has been reported by (Xia *et al*., 2004).

The results showed that the second period was more effective in causing most types of sperm abnormalities. Similar results were recorded by Taha (2000) when he studied the effect of different periods on sperm abnormalities in male mice treated with the pesticide Raxil. As well our results were similar to those reported by Karim (1993) when he studied the effect of different periods on sperm abnormalities in male mice treated with cyclophosphamide. Increasing of the abnormalities especially head abnormalities in the sperm of the second period indicates that the toxicity of this substance is accumulating in the body of the animal. It seems that the animal cannot detoxify the adverse effect of Cyren easily in short time.

Table 2A: Mean ± S.E for the Effects of Cyren (Doses, Periods and their Interactions) on Sperm Abnormalities of Male Albino Mice

Factors		Sperms with abnormal heads						
		Hookless sperm	Sperms with Swollen head	Sperms with amorphous head	Sperms with Defective hook	Sperms with Banana head	Double head sperm	Total of sperms withabnormal head
Doses	D0(control)	3.20±0.48c	1.40±0.22c	9.30±0.55c	2.10±0.31c	3.50±0.31c	0.20±0.13b	19.7
	D1	11.40±1.30b	9.80±1.08b	25.50±2.78b	10.50±1.27b	7.40±1.30b	0.80±0.24b	65.4
	D2	13.60±1.29ab	11.40±1.13b	27.0±2.80b	12.50±1.50b	10.20±1.30a	2.50±0.54a	77.2
	D3	16.40±1.73a	15.20±1.07a	31.90±3.11a	15.10±1.33a	11.50±1.25a	2.80±0.55a	92.9
		p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	
Periods	P1	8.35±0.85b	7.40±1.01b	17.45±1.37b	7.40±0.88b	5.70±0.54b	0.75±0.16b	47.05
	P2	13.95±1.60a	11.50±1.43a	29.40± 2.82a	12.70±1.52a	10.60±1.12a	2.40±0.43a	80.55
		p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	
Interactions(Doses and Periods)	D0P1	3.00±0.70e	1.20±0.20d	9.00±0.70d	2.20±0.58f	3.60±0.50de	0.20±0.20b	19.2
	D0P2	3.40±0.74e	1.60±0.40d	9.60±0.92d	2.00±0.31f	3.40±0.50e	0.20±0.20b	20.2
	D1P1	8.20±0.8602d	7.00±0.70c	18.40±1.86c	7.20±1.06e	4.00±0.70de	0.20±0.20b	45
	D1P2	14.60±1.36bc	12.60±0.92b	32.60±2.50b	13.80±0.86bc	10.80±1.15bc	1.40±0.24b	85.8
	D2P1	10.20±0.86d	8.40±0.67c	19.40±1.63c	8.40±0.92de	7.20±0.86cd	1.20±0.20b	54.8
	D2P2	17.00±1.00ab	14.40±0.92ab	34.60±1.96ab	16.60±0.92ab	13.20±1.56ab	3.80±0.66a	99.6
	D3P1	12.00±0.70cd	13.00±1.00b	23.00±1.64c	11.80±0.86cd	8.00±0.70c	1.40±0.24b	69.2
	D3P2	20.80±1.82a	17.40±1.32a	40.80±1.15a	18.40±1.36a	15.00±0.70a	4.20±0.58a	116.6
		p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	

Note:Similar letters in each column refer to non significant difference btween maens while different letters refer to a significant difference between them. (Duncan, 1955)

Table 2B: Mean ± S.E for the Effect of Cyren (Doses, Periods and their Interactions) on Sperm Abnormalities of Males Albino Mice

Factors		SpermsTail Abnormalities				
		sperms with Double tail	Sperms withbent midpiece defect	Sperms with Coiled tail	Total of sperms with abnormal tail	Total of abnormal sperms
Doses	D0(control)	0.10±0.10b	16.30±0.81c	2.30±0.30c	18.7	38.4
	D1	0.60±0.16b	31.10±2.55b	12.40± 1.30b	44.1	109.5
	D2	0.80±0.29ab	35.40±3.00ab	16.90±2.17a	53.1	130.3
	D3	1.50±0.26a	36.80±2.81a	17.50±2.18a	55.8	148.7
		p<0.01	p<0.01	p<0.01		
Periods	P1	0.40±0.11b	24.35±1.28b	8.35±0.94b	33.1	80.15
	P2	1.10±0.21a	35.45± 2.78a	16.20±2.01a	52.75	133.3
		p<0.01	p<0.01	p<0.01		
Interactions(Doses and Periods)	D0P1	0.00±0.00	16.80±1.24c	2.00±0.316d	18.8	38
	D0P2	0.20±0.20	15.80±1.15c	2.60±0.50d	18.6	38.8
	D1P1	0.40±0.24	23.80±1.28b	8.80±0.86c	33	78
	D1P2	0.80±0.20	38.40±1.07a	16.00±0.70b	55.2	141
	D2P1	0.20±0.20	27.20±1.77b	11.20±0.86c	38.6	93.4
	D2P2	1.40±0.40	43.60±1.96a	22.60±2.06a	67.6	167.2
	D3P1	1.00±0.00	29.60±1.32b	11.40±0.92c	42	111.2
	D3P2	2.00±0.44	44.00±2.81a	23.60±1.43a	69.6	186.2
		N.S	p<0.01	p<0.01		

Similar letters in each column refer to non significant difference while different letters refer to significant difference between them; N.S= Non significant

The induction of sperm abnormalities not only due to CA, it is has been argued by Rivkin*et al*. (2005) that sperm-head abnormalities may arise from point mutations rather than gross chromosomal changes who described sperm tail abnormalities including bobtail (bent mid-piece), U-shaped tail, shortened and thick tail in transgenic mice by insertion of neo^r gene into the intron 6 of K9 gene. Cheng *et al*. (2007) examined the morphological defects in mice lacking the Taf71 gene. Sperm from adult epididymis were analysed and a number of sperm head bent back to the tail were observed. Also Taf71 mutant sperm were folded at the proximal middle piece in comparing to the wild type sperm. Therefore, the results of the two previous reports confirm that the bent mid piece defect might be of a genetic origin. These postulations coincides with the results obtained from Cyren pesticides in this study which is correlated to a significant increase of bent mid piece defect and administration of the three doses of the pesticides used in this work.

Conclusions and recommendations

We can conclude from the experimental results that Cyren which is widely used in Kurdistan Region of Iraq is a mutagenic substance. For this reason, strict limitations should be put on its use, other pesticides as well should be handled with care as it has been reported by Sanborn *et al.* (2004) that most pesticides have adverse effects on human

health and environments. We also should look for alternatives to these pesticides with none or very low toxic effects on human and environments and banning these pesticides when it is possible. More study should be done at molecular level to understand the effectsof these pesticideson DNA and genes.

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