TOXIC EFFECTS OF MILLER ON CHROMOSOMES AND SPERMS OF ALBINO MALES MICE

Yousif. M. Fattah[1] and Sarbast. I. Mustafa[2]
[1] Department of Biology, Faculty of Science, University of Zakho, Kurdistan Region - Iraq.
[2] College of Agriculture, University of Dohuk, Kurdistan Region - Iraq.
(Accepted for publication: September 9, 2013)

Abstract

The genotoxicity of Miller (glyphosate) herbicide was evaluated in males albino mice *Mus muscles* BALB/c strain. The tested parameters were chromosomal aberrations and sperm abnormalities to evaluate a possible damage effects on genetic material and sperms. The herbicide was administered orally to adult male laboratory mice in 4 different doses (0, 250, 500, and 750 mg/kg of body weight). The mice were killed at two periods: after 3 and 6 weeks. The results of chromosome aberration assay revealed that all the tested doses and periods induced structural chromosomal aberrations (CAs) such as centromeric gaps, chromatid gaps, delations, dicentric chromosomes and ring chromosomes. The results of sperm abnormality assay revealed that miller has the ability to induce sperm abnormalities in all doses used comparing to untreated mice, which represented by hookless sperm, swollen head sperm, amorphous head sperm, sperm without head, defective hook sperm, banana head sperm, double head sperm, sperm without tail, double tail sperm, bent midpiece defect and coiled tails.

Key words: glyphosate, chromosome aberrations, sperm abnormalities, herbicide.

Introduction

Pesticides, including herbicides, insecticides, and fungicides are used extensively to improve crop yields and as a result, they accumulate in the environment and human unavoidably exposed to them (Van der Werf, 1996). Studies show that toxic residues may rise about ten times through the food chain due to biological magnification in the body fat of living organisms and becoming more concentrated as they move from one creature to another, because living organism cannot get rid of these toxins by their biological metabolism (Cornell university: pesticide fact sheets, 2007). Because of their biological activity, the indiscriminate use of pesticides may cause undesired effects to human health, for instance, the induction of DNA damage can potentially lead to adverse reproductive outcomes, or induction of cancer, and many other chronic diseases (Lander *et al.,* 2000 and Zhao *et al.*, 2002). Most of pesticide had been tested for their genotoxicity and carcinogenicity using different testing assays.

The United States Environmental Protection Agency (U.S EPA 1993) classified glyphosate as Group E, evidence of non carcinogenicity in humans, based on studies of laboratory animals that did not produce compelling evidence of carcinogenicity. Most investigations in lab animals presented in glyphosate Technical Fact Sheet published by National Pesticide Information Center (Miller *et al*, 2010) confirmed non-carcinogenicity effect of glyphosate in the low-doses and mid-doses, but in high-doses, slightly increased incidence of renal tubular adenomas, increased incidence of hepatocellular hypertrophy, hepatocellular necrosis and interstitial nephritis. However the EPA and an independent group of pathologists and biometricians concluded that the occurrence of adenomas was not caused by glyphosate, as well glyphosate was negative in various *in vivo* and *in vitro* test systems evaluating gene mutation, chromosomal aberration and DNA damage (Cal/EPA, 1992).

Miller or glyphosate [N-(phosphonomethyl) glycine - isopropylamine (IPA) salt; $C_3H_8NO_5P$] is a post-emergent, non-selective phosphonoglycine herbicide. In 1970 it was discovered to be a herbicide by the chemist John E. Franz of Monsanto company,USA. In 2001 considered the most heavily used herbicide on crops, in forestry and in residential settings. In plants, glyphosate disrupts the shikimic acid pathway through inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate(EPSP) synthase. The resulting deficiency in EPSP production leads to reductions in aromatic amino acids that are vital for protein synthesis and plant growth (Vencill, 2002). The shikimic acid pathway is specific to plants and some microorganisms. The absence of this pathway in mammals may explain the low toxicity of glyphosate to non-target organisms(WHO 1996). Glyphosate is not absorbed by a plant's root system because of its strong adsorption to the

soil. However, it is easily absorbed by leaves from spray residues and translocated throughout the plants and fruits. Therefore, glyphosate concentration may increase in plants immediately after spray. Lettuce, carrots, and barley contained glyphosate residues up to one year after the soil was treated with 3.71 pounds of glyphosate per acre (McMullan, Honeggar, and Logusch, 1990). In soils, glyphosate is readily degraded by soil microbes to inorganic constituents, including carbon dioxide and phosphate. However, no appreciable degradation of glyphosate was observed in water via chemical, microbiological or photolytic processes for 78 days (Anton *et al.*, 1993). Chromosome aberrations, sister chromatid exchange (SCE), and mitotic index were studied in peripheral lymphocytes from three healthy donors exposed *in vitro* to different concentrations of glyphosate. Glyphosate produced a dose-related increase in the percent of aberrant cells and an increase of SCE per cell, but did not affect the mitotic index (Lioi,*et al.* 1998). In another research, Prasad *et al.,*(2009) observed that Glyphosate treatment significantly increased chromosomal aberrations (CAs) and micronuclei (MN) in bone marrow cells of Swiss albino mice. Poletta *et al*. (2009) evaluate the genotoxic potential of glyphosate in erythrocytes of broad-snouted Caiman (*Caiman latirostris*) using Comet assay and MN test. A significant increase in DNA damage was observed at a concentration of 500 µg/egg or higher, compared to untreated control animals. Results from both the Comet assay and MN test revealed a concentration-dependent effect.

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). Chlorpyrifos at dose levels of 7.5, 12.5 and 17.5 mg/kg BWT/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 reduction in sperm count and thus affect fertility (Joshi *et al*., 2007). Clair *et al.* (2012) found that glyphosate at higher doses provoke necrosis and apoptosis in germ cells and membrane degradation in Sertoli /germ cells as well decrease testosterone by 35% at lower doses.

In the literature there are a lot of other examples showing the adverse effects of different types of pesticides on the genetic materials and sperms of different mammals. In contrast to these data Bayer Glyphosate 450 Herbicide Product safety sheet data (2010) indicate that this herbicide is practically non-toxic to fish, aquatic invertebrates, birds and bees and has low toxicity to earthworms. Dietary concentrations of up to 10,000 ppm or 293 mg/kg/day of glyphosate given to rats over two generations had no effect on male or female sexuality and fertility. The lowest observed adverse effect level (LOAEL) for parental and offspring toxicity is 3000 ppm, based upon a reduction of body weight at 10,000 ppm (Moxon, 2004). Spermiotoxicity study by Akcha *et al.* (2012) revealed that glyphosate had no cytotoxic effects on oyster spermatozoa.

These data presents inconsistent and conflicting results on the effects of glyphosate. A report of Buffin and Jewell (2001) on health and environmental impacts of glyphosate as well had included a lot of divergence and controversial data. The aim of this research is to investigate the effects of glyphosate (Miller) on chromosomes and sperms of mice to clarify some of these conflicts.

Material & Methods

Reagents

Miller, in the form of Glyphosate 480 g / Liter was brought from local markets of Dohuk city. The acute oral LD_{50} of Miller to male albino mice was found to be 1568 (mg/kg bw) (NTP, 1992). Miller was used in three doses (250, 500, and 750) mg/kg of body weight. The doses were prepared by diluting the herbicide with distilled water then placed in clean and dry bottles. The mice were treated orally 3 times weekly by using dosage syringe prepared locally from 2 ml disposable syringe and needle.

Animals

Adult males of Swiss albino mice (*Mus musculus*) BALB/c (8-10) weeks in age, weighing (30-35) gm were used 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).A standard diet and water was used to feed the mice.

Preparation of chromosomes 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).The slides were air dried at room temperature, stained with 2 % Giemsa 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 and transferred 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, then 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).

Experimental design and Statistical analysis

The experiment was carried out in a factorial design arranged in a Completely Randomized Design (CRD) .The main factors were: **A-** Miller doses, the concentrations were (0.0, 250, 500, and 750 mg/kg b.w.). Each treatment was repeated 5 times. **B-** Periods (P_1 =3weeks, P_2=6 weeks).

Statistical analyses were performed with SAS software. Data were analyzed using one way analysis of variance (ANOVA) followed by Duncan's multiple range of the doses and periods (Duncan, 1955). Results were reported as mean values ± S.E. and differences were considered as significant at ($P \leq 0.05$).

Results

Effects of Miller on chromosomes of bone marrow cells of mice

The frequencies of different types of structural chromosomal aberrations (CA) induced by the herbicide miller in bone marrow cells of mice are shown in Table (1). The results show a significant 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 are shown in Figure 1. The data in Table 1 also reveal great differences in the total percentage of aberrations. These values increased with increase of miller doses as well with extending of the treatments period. The results in Table 1 indicate that the most types of aberrations were the centromeric gaps and ring chromosomes at D3 5.60±0.47 and 7.20±0.71 respectively when compared to control D0 2.50±0.42 and2.10±0.23, respectively. The least CA was the dicentric chromosomes in control group 0.30±0.15 increased to 0.60±0.16 in D3.

The number of CA increased with extending of the doses period as shown in table (1). There was a highly significant effects ($p<0.01$) of periods on centromeric gaps, chromatid gaps and ring chromosomes and significant effects ($p<0.05$) on chromatid deletion, while the periods didn't shows any effects on the formation of dicentric chromosomes.

Significant interactions ($p<0.05$) between doses and periods has been found in ring chromosomes while interaction in the rest of the parameters were non significant (Table 1).The highest interaction value scored in D3P2 8.80±0.86 for the ring chromosome. The data in table (1) as well shows that the total values of interactions increased with the increase of miller doses as well with extending of the treatments period.

Table (1): Mean values of different chromosomal aberrations induced by miller in bone marrow cells of Swiss male mice

Factors		Chromosome Aberrations					
		Centromeric gap	Chromatid delation	Chromatid gaps	Dicentric chromosome	Ring chromosome	Total-percent aberrations
Doses	D0(control)	2.50±0.42b	0.20±0.13b	1.10±0.10b	0.30±0.15	2.10±0.23c	4.1
	D1	4.60±0.37a	1.30±0.26a	3.40±0.37a	0.20±0.13	4.70±0.39b	9.5
	D2	4.70±0.44a	1.50±0.22a	3.80±0.41a	0.40±0.16	5.40± 0.30b	10.4
	D3	5.60±0.47a	2.00±0.29a	4.00±0.42a	0.60±0.16	7.20±0.71a	12.2
Significant		p<0.01	p<0.01	p<0.01	N.S	p<0.01	
Periods	P1	3.90±0.35b	1.00±0.17b	2.55±0.27b	0.35±0.10	4.10±0.36b	11.9
	P2	4.80±0.40a	1.50±0.24a	3.60±0.39a	0.40±0.11	5.60±0.59a	15.9
Significant		p<0.05	p<0.05	p<0.01	N.S	p<0.01	
Interactions(Doses and Periods)	D0P1	2.40±0.60	0.20±0.20	1.00±0.00	0.40±0.24	2.00±0.31d	6
	D0P2	2.60±0.67	0.20±0.20	1.20±0.20	0.20±0.20	2.20±0.37d	6.4
	D1P1	4.20±0.58	1.00±0.31	2.80±0.37	0.20±0.20	4.00± 0.44c	12.2
	D1P2	5.00±0.44	1.60±0.40	4.00±0.54	0.20±0.20	5.40±0.50bc	16.2
	D2P1	4.20±0.66	1.20±0.20	3.20±0.58	0.40±0.24	4.80±0.37bc	13.8
	D2P2	5.20±0.58	1.80±0.37	4.40±0.50	0.40±0.24	6.00±0.31b	17.8
	D3P1	4.80±0.66	1.60±0.40	3.20±0.37	0.40±0.24	5.60±0.50b	15.6
	D3P2	6.40±0.50	2.40±0.40	4.80±0.58	0.80±0.20	8.80±0.86a	23.2
Significant		N.S	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.

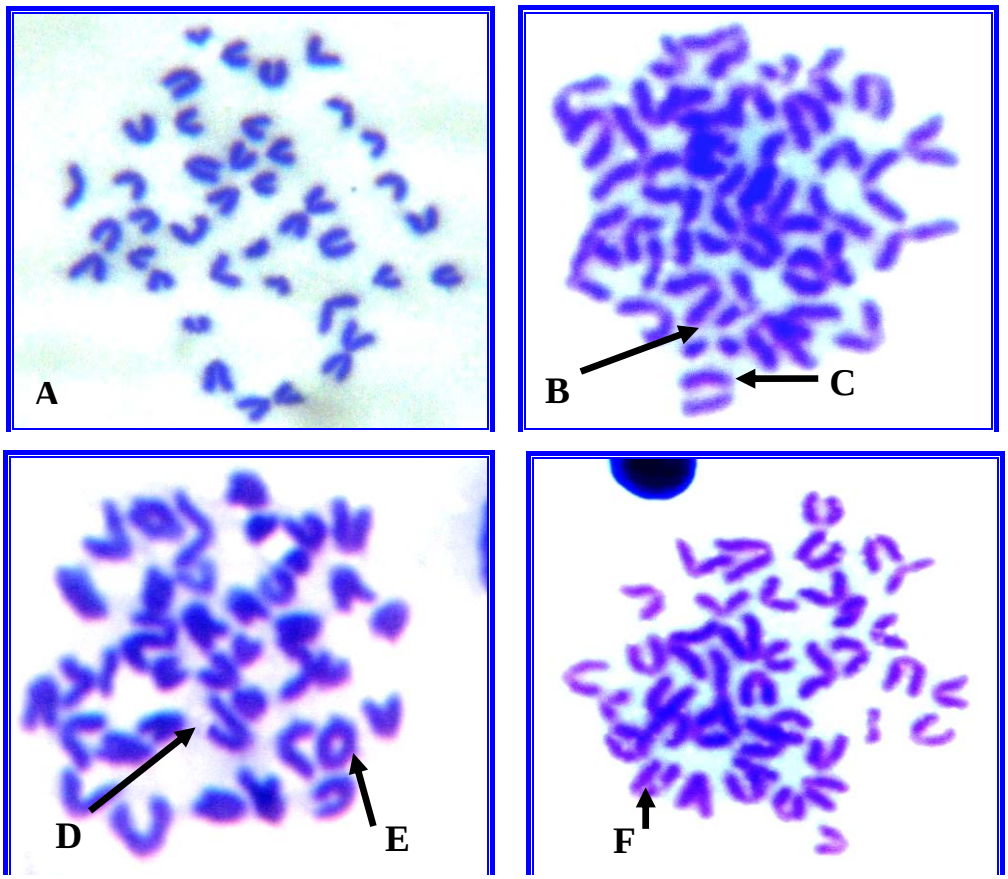

Figure (1): Different types of chromosomal aberrations induced by miller herbicide in bone marrow cells of Swiss albino males mice **(100x).** **A-** normal chromosomes; **B-** centromeric break; **C-** centromeric gap; **D-** Deletion **E-** Ring Chromosome; **F-** Chromatid gap.

Effects of Miller on sperms of mice

The data in tables (2A and B) represent the effects of miller on the sperms of Swiss albino male 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 is obvious that there is a high significant difference in all types of sperm abnormalities listed in the tables due to the effects of the treatments when compared to control. The total number of abnormal sperms increased constantly with increase of miller doses. The highest total number of abnormal sperms was in D3 = 120.7. The highest mean value of abnormal sperms head in treated animals (Table 2.A) compared to other types of head abnormalities was 27.00±2.43 in amorphous head sperm. The highest mean value of abnormal sperms tail (Table 2.B) compared to other types of tail abnormalities was 30.80±1.90 in bent mid piece defect. The least affected trait was the sperms with swollen head with value of 1.40±0.22 in D0 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 (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 non affected traits were Sperms with Banana head, Sperms with Double heads and Sperms with Double tail. The highest value of total abnormal sperm was in D3P2 with value of 142.4, while the least interaction was in D0P1 with total abnormal sperms 38. The most interaction between the periods and doses was in D3P2 with value of 35.40±1.72 scored in sperms with bent mid piece defect, while no interactions in D0P1 for sperms with double tail.

Most types of sperms abnormalities in males of albino mice induced by Miller herbicide are shown in Figure (2).

Table (2.A): Mean values of different abnormalities in sperms heads of Swiss albino mice induced by miller

Factors		Sperms Head Abnormalities						
		Hookless sperms	Sperms with Swollen head	sperms with amorphous head	Sperms with Defected hook	Sperms with Banana head	Sperms with Double heads	Total of sperms with abnormal heads
Doses	D0(control)	3.20±0.48c	1.40±0.22c	9.30±0.55c	2.10±0.31c	3.50±0.34c	0.20±0.13	19.7
	D1	10.60± 0.92b	10.20±0.72b	18.80±1.48b	5.90±0.67b	5.90±0.58b	0.20±0.13	51.6
	D2	11.10±0.87b	9.90±0.91b	20.10±1.44b	6.60±0.66b	7.60±0.54ab	0.10±0.10	55.4
	D3	16.40±1.43a	14.00±0.64a	27.00±2.43a	9.40±0.68a	9.00±0.57a	0.30±0.15	76.1
	significant	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	N.S	
Periods	P1	9.250±0.99b	8.10±1.14b	15.25±1.03b	5.25±0.55b	5.80±0.50b	0.150±0.08	43.8
	P2	11.40±1.46a	9.65±1.14a	22.35±2.07a	6.75±0.83a	7.20±0.62a	0.250±0.09	57.6
	significant	p<0.01	p<0.05	p<0.01	p<0.01	p<0.01	N.S	
Interactions(Doses and Periods)	D0P1	3.00±0.70c	1.20±0.20e	9.00±0.70e	2.20±0.58	3.60±0.50	0.20±0.200	19.2
	D0P2	3.40±0.74c	1.60±0.40e	9.60±0.92e	2.00±0.31	3.40±0.50	0.20±0.200	20.2
	D1P1	12.00±1.30b	9.40±1.24cd	15.20±1.06d	5.00±0.70	4.80±0.66	0.20±0.200	46.6
	D1P2	9.20±1.06b	11.00±0.70bc	22.40±1.53b	6.80±1.06	7.00±0.70	0.20±0.200	56.6
	D2P1	9.00±0.70b	7.60±0.60d	16.60±1.20cd	6.00±0.70	6.80±0.86	0.00±0.00	46
	D2P2	13.20±0.86b	12.20±0.86ab	23.60±1.32b	7.20±1.15	8.40±0.50	0.20±0.200	64.8
	D3P1	13.00±1.00b	14.20±1.06a	20.20±0.86bc	7.80±0.58	8.00±0.70	0.20±0.200	63.4
	D3P2	19.80±1.59a	13.80±0.86a	33.80±1.65a	11.00±0.70	10.00±0.70	0.40±0.24	88.8
	significant	p<0.01	p<0.05	p<0.01	N.S	N.S	N.S	

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

Table (2.B): Mean values of different abnormalities in sperms tail of Swiss albino mice induced by miller

Factors		Sperms Tail Abnormalities				
		Sperms with Double tail	Sperms with bent midpiece defect	Sperms with Coiled tail	Total of sperms With abnormal tails	Total no. of abnormal sperm
Doses	D0(control)	0.10±0.10b	16.30±0.81b	2.30±0.30c	18.7	38.4
	D1	0.50±0.22ab	30.10±2.34a	7.50±0.52b	38.1	89.7
	D2	0.40±0.16ab	28.30±1.94a	7.80±0.66b	36.5	91.9
	D3	1.10±0.31a	30.80±1.90a	12.70±1.36a	44.6	120.7
significant		p<0.01	p<0.01	p<0.01		
Periods	P1	0.200±0.09b	22.550±1.07b	6.000±0.65b	28.75	72.55
	P2	0.850±0.19a	30.200±2.03a	9.150±1.17a	40.2	97.8
significant		p<0.01	p<0.01	p<0.01		
Interactions(Doses and Periods)	D0P1	0.00±0.00	16.80±1.24c	2.00±0.31d	18.8	38
	D0P2	0.20±0.20	15.80±1.15c	2.60±0.50d	18.6	38.8
	D1P1	0.20±0.20	23.80±1.93b	6.80± 0.80bc	30.8	77.4
	D1P2	0.80±0.37	36.40±1.07a	8.20±0.58bc	45.4	102
	D2P1	0.20±0.20	23.40±1.36b	6.20±0.66c	29.8	75.8
	D2P2	0.60±0.24	33.20±1.77a	9.40±0.50b	43.2	108
	D3P1	0.40±0.24	26.20±1.65b	9.00±0.70b	35.6	99
	D3P2	1.80±0.37	35.40±1.72a	16.40±1.02a	53.6	142.4
significant		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.

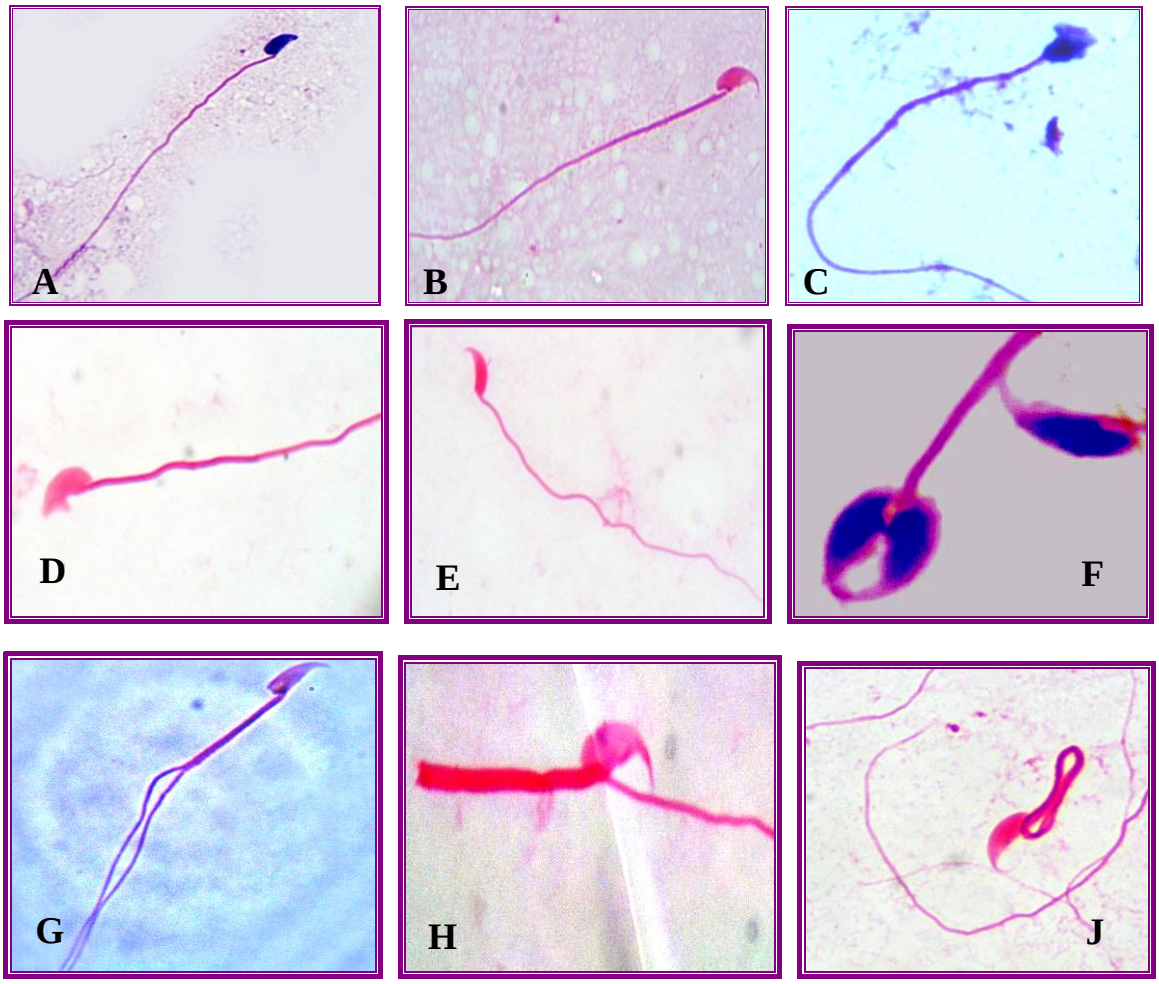

Figure (2): Different types of mice sperms abnormalities induced by miller herbicide (100x). A- hookless sperms; B- swollen head sperm; C-sperm with amorphous head; D-sperms with defected hook; E- sperms with banana head; F- Sperm Double heads; G- Sperms with Double tail; H- Sperms with bent midpiece defect; J- Sperms with Coiled tail.

Discussion

Effects of Miller on chromosome of bone marrow cells of mice

Miller or glyphosate, being one of the most commonly used herbicides, has been extensively studied over the last 20 years. Literature on cytotoxic and genotoxic activity glyphosate is abundant but it has included a lot of controversial data (Buffin and Jewell, 2001). In the present study, the genotoxicity of miller was revaluated. The experimental data revealed significant differences between the doses in their effects on all types of chromosomal aberrations except the dicentric chromosome. Percentage of chromosomal aberrations increased with increasing dose concentration. These results are smiler to those reported by Prasad *et al.*, (2009), in which glyphosate were administered by a single intraperitoneal dose (25 and 50 mg/kg b.wt) to males of albino mice and they found that glyphosate significantly increase chromosomal aberrations in both treatment and time compared to the control. They attributed it either to induction of DNA lesions or interference of glyphosate with DNA repair. Peluso *et al.*,(1998) also reported a dramatic increase in the number of oxidized guanine, 8-hydroxylguanine (8-OHdG), residues in the DNA of liver cells isolated from mice treated with Glyphosate which also may be the reason of chromosomal aberrations in bone marrow cells of mice as observed in the current study. Concurrently, Bhunya and Behera (1988) observed similar increase in chromosomal aberrations in bone marrow cells of Swiss albino mice when they used Monocrotophose pesticide in their experiments. These results also agree with those reported by Taha, (2000) who showed that exposure to granstar herbicide induce chromosomal aberrations in all doses and

periods. As well same results were obtained by Jha *et al*., (2002) who explained that increasing dose of carbazole lead to increase the frequency of some chromosomal aberrations per cells in bone marrow cells of albino mice.

The present study show significant differences between periods in their effects on the increase of chromosomal aberrations except for dicentric chromosome aberration. These results are supported by Sulaiman, (2000) who observed that chromosomal aberration is dependent on the duration of the study when he studies the genetic and histological effects of cigarette smoke on albino mice. Similar results were also obtained by Holden *et al*., (1973) who showed that increasing exposure time would increase the rate of chromosomal aberrations in bone marrow cells of CD-1 albino mice treated with 6-mercapto purine. In contrast to these results Kourakis *et al*., (1992) observed no positive correlation between the frequency of chromosomal aberrations and the duration of exposure in peripheral blood lymphocytes of workers occupationally exposed to mixture of pesticides. The present study revealed that some types of aberration increased with increasing exposure time, which might be due to that some cells exposed to harmful effects at any phases of the cell cycle, also the chromosomal density in these stages are the highest.

The interactions between doses and periods and their effects on chromosomal aberrations show no significant effects on all types of chromosomal aberrations except for ring chromosome aberration.

Effects of miller on sperms of mice

The result of the current study shows that different doses of Miller caused highly significant differences in all types of sperm abnormalities except double head sperms abnormality. The third dose was the most effective in causing most types of sperms abnormalities. These results are similar to those reported by El-Nahas *et al.* (1989) who explained that Curacron (an organophosphorus pesticide) caused different morphological sperm abnormalities which increased significantly after treatment with Curacron. The increase was dose-dependent. Similarly, Mathew *et al.* (1992) observed that Methyl-parathion showed a dose related increase in the percentage of sperm abnormalities. The current results also agree with the findings reported by Yousef *et al*. (1995) who explained that glyphosate showed decline in body weight, libido, ejaculation volume, sperm concentration and increases in the abnormal and dead sperms. Hurtado de Catalfo Graciela *et al*. (2011) as well noticed alterations in spermatozoa morphology and in plasma membrane integrity when they tested dimethoate, glyphosate and zineb. Radioimmuno assay analyses (RIA) demonstrated androgenic hormone imbalance in plasma and testes. The acrosome reaction was also altered. Free thiols (positively correlated with DNA denaturation) and fructose levels were elevated in seminal vesicles from treated rats. Taking into account the low doses of pesticides that provoke these alterations, it was assumed that the environmental pollution may play a key role as a causative factor for sperms abnormalities. These results are another support to the present study. Wyrobek *et al.* (1975) mentioned that the chromosomal aberrations may cause abnormal sperm morphology in a number of ways. One possibility is that the presence of a translocated chromosome within the germ cell will lead to the malformation of the sperm head. A second possibility is that chromosomal imbalance, i.e., aneuploidy, duplications or deficiencies, within the spermatid or haploid cells causes abnormalities in shape. These notes may explain the formation of abnormal sperms in the current study.

Periods showed significant effects on all types of sperms abnormalities except double head sperm abnormality. Furthermore, the results showed that the second period was highly effective in inducing most types of sperm abnormalities. Similar results were observed by Taha, (2000) who explained that the periods had significant effects on sperm abnormalities when he studied the effect of different periods on sperm abnormalities in male mice treated with the herbicide granstar.

In this study, the interaction between doses and periods and their effects on sperm abnormalities revealed significant differences in most types of sperm abnormalities. Since the process of spermatogenesis is genetically controlled, the abnormalities in sperms reflect the abnormalities in chromosomes of spermatogenic cells. The spermatogenesis is a continuous process and the inducing agents may act at various steps of spermiogenesis. This may be the reason of the inconsistence response to glyphosate doses and periods which led to significant differences due to the interaction

between doses and periods in most of sperm parameters.

References

Akcha F., C.Spagnol and J.Rouxel(2012). Genotoxicity of diuron and glyphosate in oyster spermatozoa and embryos. *Aquat Toxicol*. 15,106-107:104-13.

Anton.F.A., L.M.Cuadra., P.Gutierrez., E.Laborda and P. Laborda (1993). Degradation behavior of the pesticides glyphosate and diflubenzuron in water. *Bull. Environ. Contam. Toxicol.* 51:881-8.

Bhunya.S. and B.Behera (1988). Studies on the genotoxicity of monocrotophose (Astaf) im mammalian in vivo system. *Mut. Res.* 223: 287-293.

Buffin.D. and T.Jewell (2001). Health and environmental impacts of glyphosate: The implications of increased use of glyphosate in association with genetically modified crops. Pesticide Action Network UK. Edited by Pete Riley, Mary Taylor, Emily Diamand and Helen Barron

Cal/EPA (1992). Summary of toxicology data, glyphosate (isopropylamine salt). California Department of Pesticide Regulation, California Environmental Protection Agency, RP-11/3/92.

Clair, E., R.Mesnage, C.Travert and GÉ.Séralini (2012). A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells in vitro and testosterone decrease at lower levels. *Toxicol In Vitro*. 26(2):269-79

Cornell University (2007). Pesticides in the environment. Pesticide fact sheets and tutorial, module 6. Pesticide Safety Education Program.

Duncan,D.B.(1955). Multiple range and multiple F tests. Biometrics11:1–42.

El-Nahas.S.M., M.H.A. De-Hondt and H.E.Abdou (1989). Chromosome aberration in spermatogonia and sperm abnormalities in curacron-treated mice. *Mut. Res*. 222(4): 409-414.

Evans, E.P., G. Breckon and C.F. Ford. (1966). An air drying method for meiotic preparation from mammalian testis. *Cytogenetic*. 41(6): 317-321.

Holden.H.E., V.A.Ray, M.G.Wahrenburg, and J.D.Zelenski (1973). Mutagenecity studies with 6-mercapto purine. 1-Cytogenetic activity in vivo. *Mutat. Res*. 20: 257-263.

Hurtado de Catalfo Graciela, Astiz Mariana, Alaniz María J. T. de and Marra Carlos Alberto (2011).Frequently-used agrochemicals lead to functional and morphological spermatozoa alterations in rats. *Journal of Toxicology and Environmental Health Sciences* Vol. 3(7), pp. 180–192,

Jha.A.M., A.C. Singh and M.K. Bharti, (2002). Clastogenicity of carbazole in mouse bone marrow cells in vivo. *Mut. Res*. 521(1-2): 11-7.

Joshi.S.C., [ikkuR.Mathur, and N.Gulati (2007). Testicular toxicity of chlorpyrifos (an organophosphate pesticide) in albino rat, *Toxicology and Industrial Health*. 23: 439-444.

Kourakis.A., M.Mouratidou, G.Kokkinos, A.Barbouti, A. Kotsis, D. Mourelatos and J. Dozi-Vassiliades (1992). Frequencies of chromosomal aberrations in pesticides sprayers working in plastic green houses. *Mut. Res*. 279(2): 145-148.

Lander.F., L.E.Knudsen, M.O.Gamborg, H.Jarventaus, and H.Norppa (2000). Chromosome aberrations in pesticide-exposed greenhouse workers. *Scandinavian Journal of Work, Environment and Health*, vol. 26, no. 5, pp. 436–442.

Lioi. M.B, Maria R. Scarfi, Antonietta Santoro, Rocchina Barbieri , Olga Zeni, Francesca Salvemini, Dino Di Berardino, Matilde V. Ursini . (1998) .Cytogenetic damage and induction of pro-oxidant state in human lymphocytes exposed in vitro to glyphosate, vinclozolin, atrazine, and DPX-E9636. *Environ Mol Mutagen* 32(1):39-46.

Material safety data sheet (2010) Bayer glyphosate 450 herbicide. www.bayercropscience.com.au

Mathew.G., K.K.Vijayalaxmi and M.Abdul-Rahiman (1992). Methyl parathion induced sperm shape abnormalities in mouse. *Mut. Res*. 280(3): 169-173.

McMullan.P., J.Honeggar and E.Logusch (1990). Confined rotational crop study of glyphosate Part II. Quantitation, characterization and identification of glyphosate and its metabolites in rotational crops. Unpublished report no. MSL-981, 1990, submitted to U.S. Environmental Protection Agency by Monsanto Agricultural Labs. Reregistration Eligibility Decision (RED) Glyphosate; EPA.

Miller.A., J.A.Gervais, B.Luukinen, K.Buhl and D.Stone (2010). Glyphosate Technical Fact Sheet; National Pesticide Information Center, Oregon State University Extension Services.

Moxon, M. E. (2004).Glophosate acid: multigeneration reproduction toxicity in rats. Unpublished report no.

CTL/P/6332, study no. RR0784, 2000, submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland, prepared by Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. *Pesticide Residues in Food - Evaluations Part 2: Toxicological*; International Programme on Chemical Safety, World Health Organization, Food and Agriculture Organization: Rome, Italy.

NTP (1992). NTP technical report on toxicity studies of glyphosate (case # 1071-83-6) administered in dosed feed to F344/N rats and B6C3F1 mice. *NIH Publication 92-3135*.

Peluso.M., A.Munnia, C.Bolognesi and S.Parodi (1998). P32-Postlabeling detection of DNA adducts in mice treated with the herbicide Roundup. *Environmental and Molecular Mutagenesis*, vol. 31, no. 1, pp. 55 59.

Poletta, G. L., A.Larriera, E.Kleinsorge and M.D.Mudry (2009).Genotoxicity of the herbicide formulation Roundup (glyphosate) in broad-snouted caiman (*Caiman latirostris*) evidenced by the Comet assay and the Micronucleus test. Mutation Research, vol. 672, no. 2, pp. 95–102,.

Prasad.S., S.Srivastava, M.Singh, and Y. Shukla, (2009). Clastogenic Effects of Glyphosate in Bone Marrow Cells of Swiss Albino Mice. *Journal of Toxicology* Volume 2009. Proteomics Laboratory, Indian Institute of Toxicology Research, Mahatma Gandhi Marg, Lucknow 226001, India

Sharma, A.K. and Sharma, A. (1980). Chromosome techniques. (3rd ed.), Buterworths. London.

Sulaiman, K.M. (2000). The genetic and histological effects of cigarette smoke on albino laboratory mice *Mus musculus*. M.Sc. Thesis. College of education / University of Salahaddin-Erbil. Iraq

Swati.D.J.(2004). Occupational Female Reproductive Hazards. Master of Industrial Hygiene Programme, Institute of Science and Technology for Advanced Studies and Research, Science College. *Journal of Health Management*. 6 (2) 201-210.

Taha, H.M. (2000). The genetic effects of pesticides (Raxil and Granstar) on albino laboratory mice *Mus musculus*. M.Sc. Thesis. Dept. of Biol./College of education. University of Salahaddin-Erbil. Iraq.

U.S. EPA(1993), Reregistration Eligibility Decision, (RED) : *Glyphosate* EPA-738-R-93-014 U.S Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances, Office of Pesticide Programs, U.S. Government Printing Office: Washington, DC, 1993.

Van der Werf. H. M. G., (1996). Assessing the impact of pesticides on the environment, *Agriculture, Ecosystems and Environment*, vol. 60, no. 2-3, pp. 81–96,

Vencill, W. K. (2002). Herbicide Handbook, 8th ed. *Weed Science Society of America*: Lawrence, KS p 231-234.

WHO.(1996).Data Sheets on Pesticides: Glyphosate International Programme on Chemical Safety, World Health Organization, Food and Agriculture Organization: Geneva, Switzerland.

Wyrobek, A. J., J. A. Heddle, and W. R. Bruce (1975). Chromosomal abnormalities and the morphology of mouse sperm heads. *Canadian Journal of Genetics and Cytology*, 17(4): 675-681, 10.1139/g75-083

Wyrobek, A.J.(1979). Changes in mammalian sperm morphology after X-ray and chemical exposure. *Genetics* 59:105-119.

Yousef.M.I., M.H.Salem, H.Z.Ibrahim, S.Helmi, M.A.Seehyand K. Bertheussen(1995). Toxic effects of carbofuran and glyphosate on semen characteristics in rabbits. *J. Environ. Sci. Health* B 30:513-34

Zhao. S., A. Narang, J. Gierthy, and G. Eadon (2002). Detection and characterization of DNA adducts formed from metabolites of the fungicide ortho-phenylphenol. *Journal of Agricultural and Food Chemistry*, vol. 50, no. 11, pp. 3351–3358.