Micro and macro genetic damage induced by the insecticide Match in mice genome

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ABSTRACT

This work aims to disclosing the capability of the well known insecticide Match N-[2,5-dichloro-4-(1,1,2,3,3,3,-hexafluoro-propoxy)-(phenyl)amino]carbonyl]-2,6-difluorobenzamide (CA) that is widely used for controlling certain fruit and leaf caterpillars in inducing micro and macro DNA damage. To investigate its possible genotoxic effects on mammals, different short term genotoxic bioassays were selected and employed. Micro DNA damage was assayed by dominant lethals assay. Macro DNA damage was evaluated by mice bone marrow chromosomes. The effect of Match upon gene expression of serum protein, development and differentiation of sperms was precisely investigated. The frequency of dominant lethal mutations in female mice sired by males treated by Match was significantly higher than that of the control group. There was also a reduction of fertile matings in females mated 15 and 21 days after treatment of males with Match. Analysis of mice bone-marrow chromosomes showed that Match proved to be a positive clastogenic agent, since significant increases of different types of aberrations (e.g., deletions, fragments, Robertsonian centric fusion) were observed and this result was confirmed by the analysis of micronucleated polychromatic erythrocytes. The results also showed that Match has a capability to interfere with spindle fibers of mice spermatid cells, since a significant number of polyploid cells were obtained. Analysis of electrophoretic pattern of proteins indicated that Match was positive in causing changes in proteins specifically in high molecular protein patterns compared with the negative control group.

Significant changes in sperm ultrastructural were observed by employing electron microscope. These changes usually were accompanied by nuclear distortion and abnormal chromatin condensation in addition to alteration in head morphology, size, chromatin content and spermatocytes with pycnotic nucleus and alteration with irregular nuclear envelop. Also, mitochondrial abnormalities were mostly accompanied by defects in axoneme including missing of some peripheral microtubules and more commonly missing of central microtubules. In addition, multinucleate spermatids, abnormal chromatin condensation, rupture of cytoplasmic membrane were also commonly observed. Transmission electron microscopy examination of mice treated with Match also indicate that most severe abnormality was the complete disappearance of one or more of nine – fiber duplets and the disappearance of some outer dense fibers.

Key words: *Mice, insecticide, dominant lethals, chromosomal aberrations, protein, ultra structure sperms alteration.*

INTRODUCTION

The effect of environmental contaminations on human health is one of the most challenging problems that face the world today. The growing world economy and movement toward global market have driven competition in industrial and technological development at a high speed towards the betterment of mankind. However, in nearly all countries such developments have focused on increased production and economic gains before realizing their impact on the environment and human health (El-Seedy *et al.*, 2006.) High level demand and respiratory exposure to pesticides during on-farm and house use; and chronic exposure to low levels of pesticides residues in food and water represent a serious source for the induction of genetic lesions (Seehy, 2003 and Hafez *et al.*, 2004) It is taken for granted that the degree of mutagenic potentiality of environmental pollutants evaluated in one test system may or may not be the same in another; therefore testing for the induction of DNA lesions and for mutagenicity using a variety of short-term assays, has become an essential part of the toxicological evaluation of contaminants (e. g. pesticides, cosmetics, drugs, food and feed additives etc).

Evidences accumulated in the last two decades have indicated that a large number of pesticides are capable of inducing genetic damage to human as well as domestic animals and economical plant. In recent years, there has been increasing awareness of the genotoxic potential of a wide variety of chemicals to which the human population is exposed either environmentally or occupationally This awareness is paralleled by the recent development of appropriate ,sensitive and practical methods for detecting and assessing the possible genetic and biological effects of these substances.

For many years, semen analysis has been routinely performed to diagnose testicular damage and infertility in human and domestic animals (Yousef *et al.,* 1996). Since sperm samples are easily obtained and sperm morphology is rapidly quantified, these observations suggest that sperm morphology in the mouse may be an applicable screen for environmental effects on germ cells. Padmalatha *et al.*,(2001) showed that sperm head abnormalities in mammals provide a unique approach to quantify the effects of environmental agents on the germ cells. Preliminary evidence suggests that these changes represent heritable sperm shape abnormalities that can be further transmitted to subsequent generations.

The long term genotoxic assay is considered to be very expensive. Thereby, the use of well validated short- term genotoxic assays has been accepted in the evaluative purposes of an environmental agent for possible genotoxicity .

The present investigation aims at disclosing the capability of the insecticide Match in inducing micro as well as macro genetic lesions in mice genome. In order to achieve such a purpose, the following bioassays were selected and employed. These include: 1- mice bone marrow chromosomes, 2- micronucleated polychromatic erythrocytes, 3- the alteration of sperm ultrastructure employing the transmission electron microscope, 4- total proteins, and 5- the mutagenic activity of the pesticide by dominant lethal assay.

MATERIALS AND METHODS

The tested insecticide, Match, was kindly provided by the Department of Plant Protection, Faculty of Agriculture, Saba Basha, and Alexandria University. The insect-

icide common name, manufacturer, and chemical composition are shown in Table (1).

Table (1): Composition, common name and manufacturer of the tested insecticide.

Composition	*Common name*	*Manufacturers*
N-[[[2,5-dichloro-4-(1,1,2,3,3,3,-hexafluoro-propoxy)-(phenyl]amino]carbonyl]-2,6- difluoro-benzamide (CA) .	Lufenuron, SORBA,and AXOR	NOVARTIS

Experimental groups

a) Negative control group animal's received H_2O. b) Cyclophosphamide, 25 mg / kg. b.wt. was used as a positive control accor-ding to Parry and Parry (1984) and Seehy (2003).

The LD_{50} of Match (3µg / gm b.wt.) is calculated according to Brusick (1986). To investigate the acute and chronic effects of Match, three different treatments (acute, chronic and control) were used. A dose of 0.5 µg / gm b.wt. as acute dose, while for chronic dose 0.01 µg / gm b. wt. was used for three different treatment times i.e. 7, 14, 21 days.

Analysis of mice bone marrow chromosomes

Each animal had orally received the proper dose. Four animals for each dose were used. Before sacrificing, each animal was injected intravenously in tail vein with 20 µg colcemid to arrest metaphases. The bone-marrow cells were collected according to Brusick (1986). Staining was carried out using 10% Giemsa-Gurr (pH=6.8). Screening of slides for mitotic spreads was conveniently accomplished with a 25X magnification objective lens and analysis was done with a 100X objective.

Mitotic index

Animals used for this assay were not injected with colcemid. A mitotic index based on at least 4000 counted cells was recorded. The mitotic activity was estimated as the percentage of dividing cells to the total number of the examined cells (Alder, 1984).

Micronucleus test

Experimental design was carried out according to the procedure described by Brusick (1986). Bone marrow smears were performed according to Schmid (1975). Staining was done according to the method described by Gollapudi and Kamara (1979).

Sperm ultrastracture

Preparation of sperms for transmission electron microscope was carried out acco-rding to the procedure described by Reynolds (1963).

Electrophoresis

SDS Polyacrylamide-gel electrophoresis was used to fractionate proteins extracted from mice serum according to the method suggested by Studier (1973). Total proteins from parents as well as from progenies (after crosses) were extracted, over night using 0.2 M Tris HCl buffer pH (6.8) containing 2% SDS and 10% glycerol. Centrifugation was carried out at 9000 rpm for 6 minutes, then 30 µl supernatant were loaded in 12.5% acrylamide slab gels containing 10% SDS. Molecular weight was calculated using simple basic computer program analysis (GEL works ID) software UVP, GEL Documentation system using SDS molecular standard 70 L Kit (97K Da to 14 KDa, Sigma).

Dominant lethal assay

The method used was the modified dominant lethal technique of Anderson (1984). Six males were used for each dose and for control. Each of the 6 males in each treatment dose was caged with two virgin females giving a total of 72 females the total number of mated females for the control was 12. Mating was detected by daily examinations of females for vaginal plugs or a discharge of the plug

All females were sacrificed 13 days after the mid week of their caging and scored for total implants which comprised of life implants and early fetal deaths the frequency of induced dominant lethal mutations was calculated according to the method of Ehling *et al.*, (1968). The dominant lethal mutations index is given by the formula:

$$= 1- \frac{\text{live implants treated}}{\text{live implants control}} \times 100$$

Differences between the control and experimental group were analyzed by Student's t- test.

Statistical analysis

The obtained data were analyzed using F- test and χ^2– test, while the micronucleus data were analyzed according to Hart and Pederson (1983).

RESULTS AND DISCUSSION

Micro–DNA damage was investigated by Dominant lethal assay, while macro DNA damage was achieved by the analysis of mice bone marrow chromosomes and micronucleus test. The effect of Match on gene expression, development and differentiation was achieved by electrophoresis and electron microscope.

Cytological analysis

Chromosomal aberrations

The analysis of mice bone-marrow cells are given in Table (2). Five main different types of aberrations were observed. They were fragment, Robertsonian centric fusion, ring chromosome, stickiness and deletions. In the negative control group total aberrant metaphases were shown to be 13%. However, more than 50% of this percentage was caused by stickiness (8%).Using cyclophosphamide (25 mg / kg b.wt.). the total percentage of aberrant metaphase was 32%. The total aberrant metaphases without stickiness ranged from 5% in the negative control to 43% after the treatment with the chronic dose for two weeks. The tested insecticide was shown to be positive in acute as well as in chronic effects.

Treatments with positive control as well as tested doses of Match were found to be positive causing significant increases of aberrant metaphases, indicating that the level of the tested doses, proved to be a clastogenic agent. In addition, cytological examination revealed polyploidy. Such a result gave evidence that the insecticide Match is capable to interfere with spindle fibers. Plate (1) represents the positive effect of Match.

Cell proliferation

The effect of the tested insecticide upon cell proliferation was investigated by estimating mitotic activity. Data obtained from cytological observations are shown in Table (3). Mitotic index was found to be 6.8 ± 0.84 in the negative control. Using 25 mg / kg of cyclophosphamide as a positive control, mitotic index was reduced to be 1.2 ± 0.43. Mitotic index ranged from 2.4 ± 0.31 for chronic treatment (3-weeks) to 7.2 ± 1.02 for acute treatment (one-week) giving evidence that cell proliferation was affected in both acute as well as chronic treatments.

Table (2): Chromosomal aberrations in mice bone- marrow after treatment with the tested insecticide Match.

Aberration / treatment		Fragments	RCF**	Ring chromosomes	Stickiness	Deletions	Total aberrations/metaphase	
							Without stickness	With stickness
Negative control		4	2	4	8	1	5	13
Positive control		6	-	2	6	4	26	32
Acute	1st week	5	4	6	10	2	17	27
	2nd week	6	8	3	11	4	19	30
	3rd week	8	7	6	12	5	25	37
Chronic	1st week	11	6	4	10	5	26	36
	2nd week	1	-	1	2	1	43	45
	3rd week	10	4	5	8	6	25	33

* Polyploid cells were noted but not counted ** RCF Robertsonian centric fusion

Table (3): Mitotic index of mice bone- marrow after treatment with the tested insecticide Match.

Treatment		MI±S.E
Negative control		**6.8± 0.84**
Positive control		**1.2± 0.43**
Acute	1st week	7.2± 1.02
	2nd week	5.3± 0.74
	3rd week	3.5 ± 0.52
Chronic	1st week	5.2 ± 0.72
	2nd week	4.3± 0.64
	3rd week	2.4± 0.31

Table (4): Micronucleated polychromatic erythrocytes in mice bone- marrow after treatment with the tested insecticide Match.

Treatment		Number of micronucleated polychromatic erythrocyte	%
Negative control		12	0.3
Positive control		103	2.57*
Acute	1st week	28	0.7*
	2nd week	44	1.1*
	3rd week	66	1.65*
Chronic	1st week	39	0.97*
	2nd week	71	1.8*
	3rd week	82	2.1*

**Significant at 0.5 level of probability* ** Per 4000 counted polychromatic erythrocytes.*

Micronucleus test

The analysis of micro nucleated polychromatic erythrocytes of mice treated with cyclophosphamide (positive control) and different doses of Match in addition to the negative control group is shown in Table (4). All tested doses of Match were found to be positive in inducing significant increases of micronucleated polychromatic erythrocytes, giving the second evidence that the insecticide Match has clastogenic acti-vity upon mice genome. However the pos-itive clastogenic

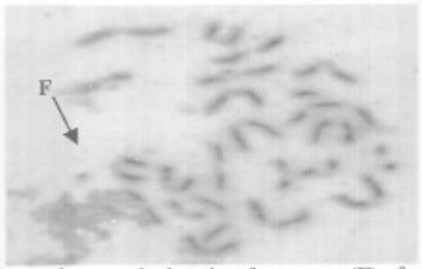
effect of the insecticide Match came also from the observation that more than one type of aberrations in a cell was obtained (Plate, 1).

Ultrastructure of sperm

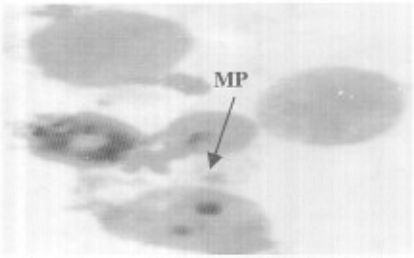
Employing electron microscope revealed frequent changes in sperm morph-ology. These changes usually accom-panied by nuclear distortion and abnormal chrom-atin condensation. In addition the altera-tions included head morphology, size, chromatin content and pycnotic nucleus with irregular nuclear envelop, (Plate, 2A). Padmalatha *et al.* (2001) and Odeigah (1997) showed that sperm shape is highly heritable, and the fraction of abnormal sperm is controlled by a multitude of autos-omal factors plus probably involvement of the sex chromosomes.

Mitochondrial abnormalities were mostly accompanied by defects in the structure of axoneme, (Plate, 2B).Axonemal alterations included missing of some peripheral microtubules and more commonly missing the central microtubules. This result is in agreement with those of Saxena *et al.*, (2004) and Miki *et al.*, (2004). They indicated that glyceraldehyde 3-phosphate dehydrogenase-S, a sperm-specific glycolytic enzyme, is required for sperm motility and male fertility. Although glycolysis is highly conserved, it is remarkable that several unique isozymes in this central metabolic pathway are found in mammalian sperm. Glyceraldehyde 3-phosphate dehydrogenase-S (GAPDS) is the product of a mouse gene expressed only during spermatogenesis and, like its human ortholog (GAPD2), is the sole GAPDH isozyme in sperm. It is tightly bound to the fibrous sheath, a cytoskeletal structure that extends most of the length of the sperm flagellum.. Furthermore, the critical role of glycolysis in sperm and its dependence on this sperm-specific enzyme suggests that GAPDS is a potential contraceptive target, and that mutations or environmental agents that disrupt its activity could lead to male infertility. Sperm axonemal alterations are known to be the primary cause of sperm immobility in man

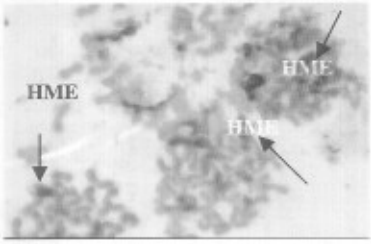
Plate (2C), demonstrates abnormal spermatocytes with altered heterochromatin content, terminalzed segregated nuclei and irregular envelop. The present results revealed distributed heterogeneously coarse chromatin in the spermatic nucleus. In addition, multinu-cleate spermatids, abnormal chromatin condensation, rupture of cytoplasmic membrane were also commonly observed (Plate 2D). Among the commonest abnormalities were the different sizes nuclei. This is in addition to presence of dense bodies inside the nuclei, (Plate 2E). The obvious relative decrease in the density of chromatin condensation seen in sperms of treated mice might be associated with chromosomal abnormalities, this may be due to a decrease in fertility potential as reported previously by Acharya *et al.* (2003) who reported that chromosomal aberrations increased sperm head abnormalities and decreased sperm count profile in lead –treated mice. Extensive mitochondrial damage was presented as extensive vaculation (plate2F). (Au *et al.*, 2000) indicating that exposure to toxicants (Cd and phenol) changed the size and shape of midpiece of spermatozoa of mussel and sea urchin, and this might affect the balance of spermatozoa in their swimming. They also observed disorganization of mitochondrial membranes and cristae, thus disrupt ATP supply for sperm movement. It is well known that ATP is essential for sperm mobility and decreased ATP equates with decreased sperm movement (Perchec *et al.*, 1995). Moreover it was also found that mitochondrial abnormalities were accompanied by flagellar degeneration in the treated samples.



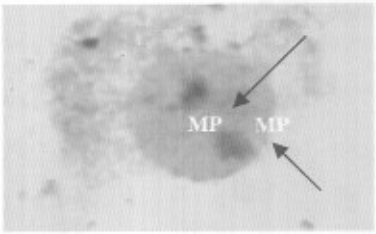
(a) Photomicrograph showing fragments (F) after treatment with the tested insecticide.



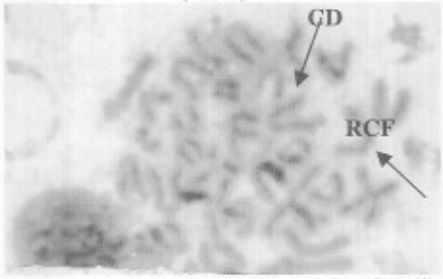
(b) Photomicrograph showing micronucleated polychromatic erythrocyte (MP).



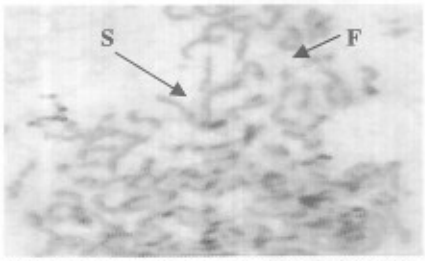
(c) Photomicrograph showing high mitotic activity (HME)



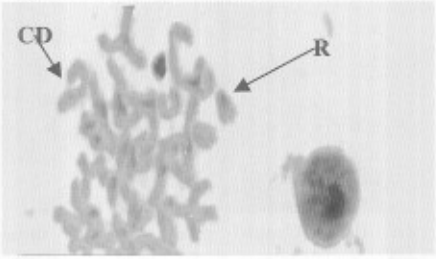
(d) Photomicrograph showing micronucleated polychromatid erythrocyte (MP).



(e) Photomicrograph showing Robertsonian Centric Fusion (RCF) and chromatid deletion (CD)

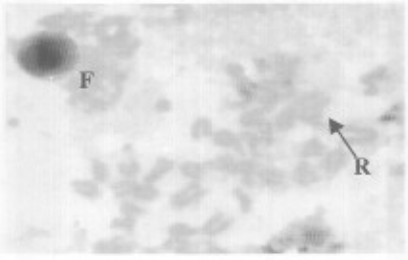


(f) Photomicrograph showing polyploidy with fragments (F) and high degree of stickiness (S).

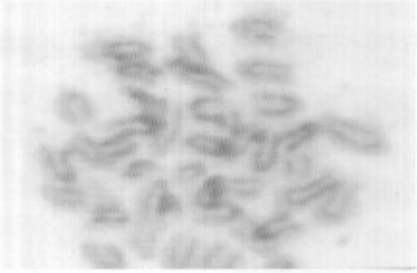


(g) Photomicrograph showing ring-chromosome (R) and chromatid deletion (CD).

(h) Photomicrograph showing chromatid deletion (CD); and fragment (F).

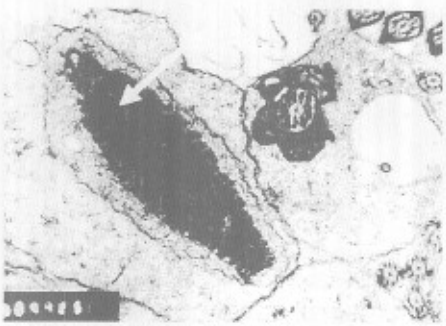


(k) Photomicrograph showing ring chromosomes (R) and fragment (F).

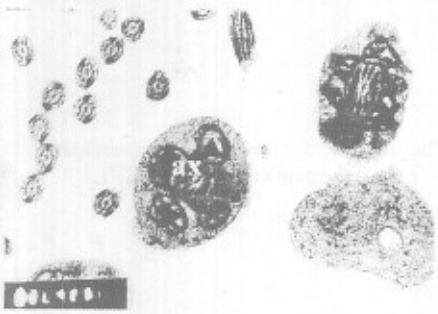


(j) Photomicrograph showing normal metaphase in negative control bone marrow

Plate (1): Giemsa–stained chromosomes from mice bone marrow cells.

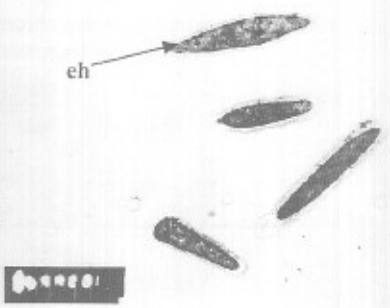


A: Electronmicrograph (E.M.) Demonstrating longitudinal section in sperm. Note: head (spindle shape) formed of dense chromatin.(arrow). X 15000).

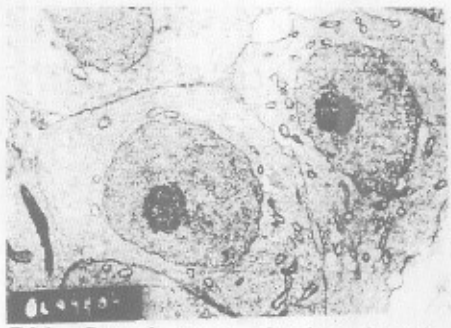


B: High power electronmicrograph control group. Illustrating sections through axoneme (ax) with normal structure (9+2) duplets (X. 15000)

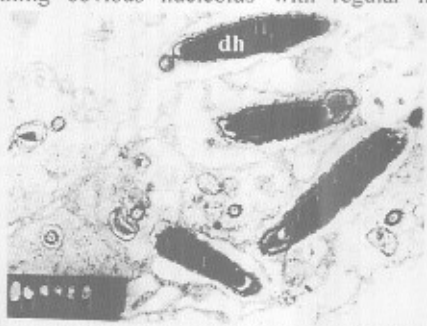
C: E.M. Showing elongated dense chromatin heads (arrows). (X 5000)



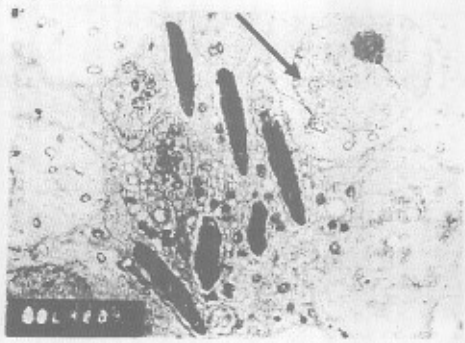
D:E.M. showing normal shaped spermatozoa with elongated heads (eh) and dense chromatin (X,



E: E.M. Control group. Section through testis demonstrating, spermatocytes with large nuclei containing obvious nucleolus with regular nuclear



F: E.M Control group. Section through testis posses spermatozoa with dense heterochromatin (dh) and normal middle piece.(X. 10000)



G: E.M. Control group. Section through testis, note spermatozoa posses corrugated plasma membrane.(X. 5000)

H: E.M Control group section through testis, showing normal shaped spermatozoa(X, 5000)

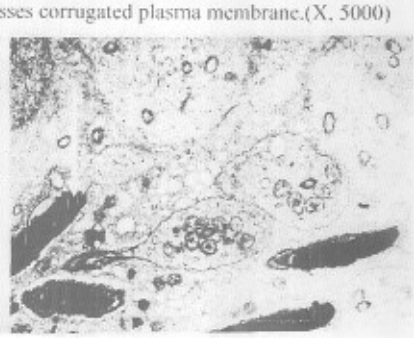


Plate (2): Electron micrographs.Treated group.Sections through tests of albino mice. (4FiG fixative, uranyl acetate.lead citrate stained preparation

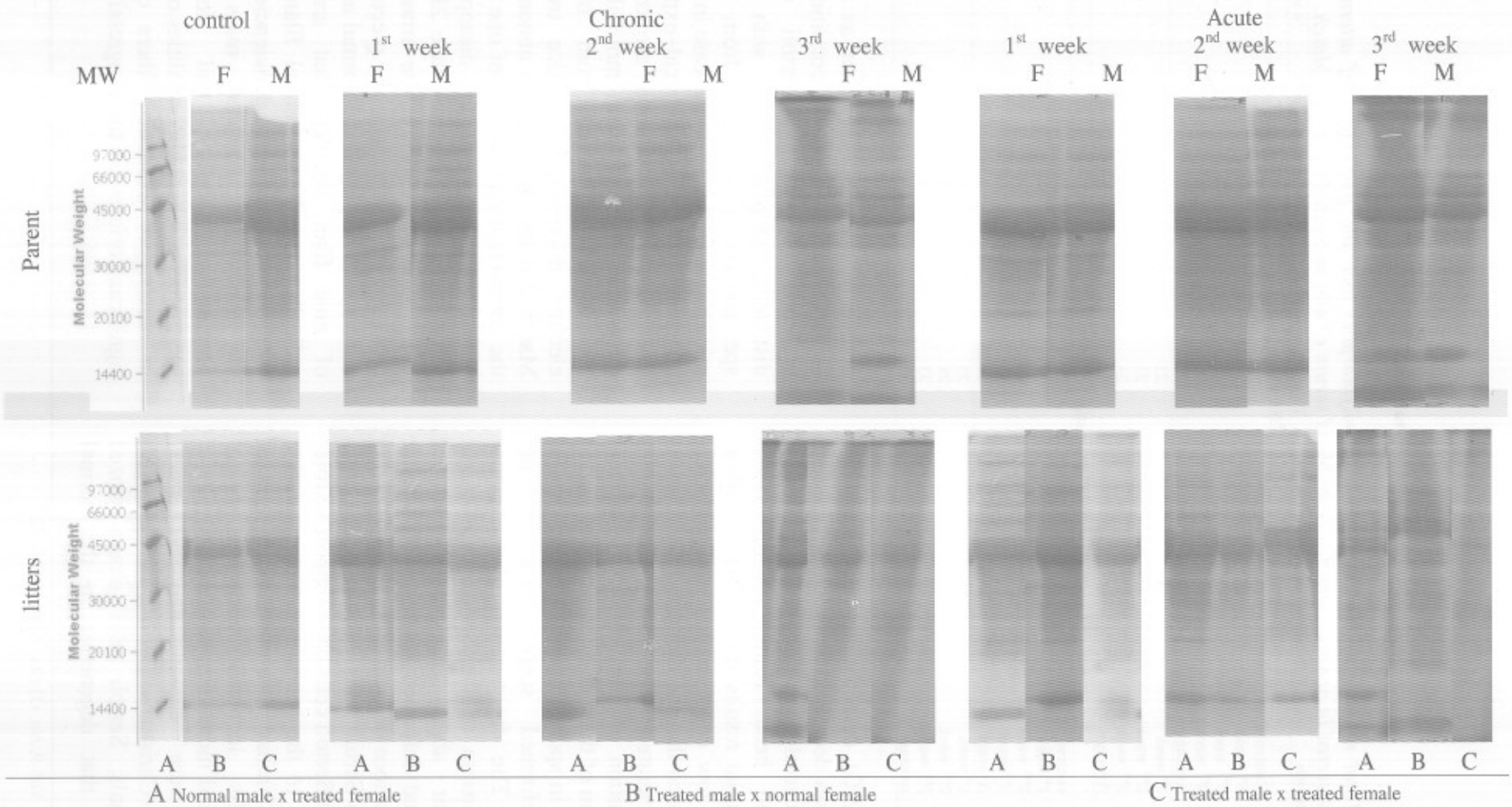


Plate (3): Eelectrophoretic patterns of protein extracted from albino treated mice.

Table (5): Total number of bands, number of polymorphic loci and percentage of alteration detected in protein extracted from blood serum of albino treated mice with Match.

Treatments		Total No. of scorable bands	Total No. of bands	No. of altered band	% of alteration
Parents					
	Control	15	29	2	13.33
Chronic	1st week	13	24	2	15.38
	2nd week	9	17	1	11.11
	3rd week	15	25	4	26.66
	Average	**12.33**	**22**	**2.33**	**17.71**
Acute	1st week	13	22	4	30.76
	2nd week	19	21	3	15.78
	3rd week	13	24	4	30.76
	Average	**15**	**22.33**	**3.6**	**25.76**
1st generation					
	control	14	28	2	14.28
Chronic	1st week	13	19	8	61.35
	2nd week	15	26	9	60
	3rd week	13	26	9	69.23
	Average	**13.66**	**26.55**	**8.66**	**63.19**
Acute	1st week	14	35	6	42.85
	2nd week	11	28	6	54.54
	3rd week	13	33	5	38.46
	Average	**12**	**32**	**5.66**	**45.28**

Plate 2(G), demonstrates section through testis pocesses decreased heterochromatin than normal, vacuolated cytoplasm, and area of lysis around head. Kamel *et al* (1997), reported similar results in *Gambursia affinis* exposed to low concentration of Bayluscide. They suggested that the deformed Sertoli cells with degenerated cytoplasmic organelles reflect cessation of metabolic processes and malformation of these cells, which may lead to disturbance in spermatogenesis in the form of altered, deformed sperm structures and orientation. The degeneration of the cytoplasmic extension of the Sertoli cells might reduce their ability in phagocytosis of abnormal and deformed sperms, which result in the appearance of greater number of sperms around each Sertoli cells. Murthy *et al* (1991) reported that Sertoli cells have supportive roles with regard to the spermatogenic maturation sequences. Similar ultrastructural changes of Sertoli cells included vacuolization of cytoplasm and increase in number and size of lysosome were demonstrated by many researchers (Christiansen *et al.*, 1998). In fish and mammals, Sertoli cells phago-cytozed germ cells that degenerate in the normal course of spermatogenesis or as a result of some deleterious agents or condition, thus the presence of multiple Sertoli cells with distended cytoplasm filled with degenerate spermatozoa in specimens from the treated mice might be due to an increase in necrosis or apoptosis induced by chemical–exposed mice. Sperm axonemal alterations are known to be the primary cause of sperm immobility in man.

It was observed from transmission electron microscopy of mice treated with Match that the most severe abnormality was the complete disappearance of one or more of nine–fiber duplets and the disappearance of some outer dense fibers, (Plate 2H). On the other hand the ultrastructure arrangement of cross section in the principle piece of normal mice sperm displayed the normal arrangement of nine fiber doublets that are arranged radically around two central filaments (9+2 arrangement). Axonemal deficiencies are often the cause of lowered mass motility, progressive motility and fertility in spermatozoa, where abnormalities or reduction in number of axonemal fibers could have significant effects on the progressive motility

of the spermatozoa; these findings could confirm the present observations.

Transcriptional factors, proteins that regulate the activity of RNA polymerase are central to the regulation of gene expression. Leucine zipper, one of these transcription factors is rich in basic amino acids (Lysine and arginine) and contains four or five leucine residue spaces at intervals of seven amino acid (Cooper, 1997)

Recently, the disappearance of some outer dense fiber has been reported to be correlated with reduced level of Lysine, Arginine and Leucine (Fouad, 2003). These amino acids which represent the main contributions of transcription factors (Leucine zipper) seem to hinder the synthesis or function of this transcription factor which in turn disturbs the regulation of gene expression. Due to the importance of leucine zippers in sperm tail outer dense fiber protein interaction, the reduction in the level of lysine, arginine and leucine could explain and might be the reason for the observed disappearance of the outer dense fiber.

On the other hand, the ultrastructure of control mice sperm revealed normal appearance of cytoplasmic membrane, mitochondrial sheath, outer dense fibers and axoneme (Plate3 A,B,C,D,E,F,G and H).

Blood Serum Protein

Electrophoretic patterns of proteins extracted from blood serum of mice treated with the tested insecticide Match are illustrated in Plate (4) and Table (5). The protein fractions were found to be distributed along a wide range of molecular weights. The total number of protein bands showed alteration value of 25. While males and females parents of the original population treated with Mach exhibited some changes in protein banding, average percentage of alteration were 17.71 and 25.76 for chronic and acute treated parents, respectively. Testing variations in protein banding patterns in the F1's revealed a sizable variation, average percentages for alteration in litters produced from chronic treated parents were 63.19 versus 45.28 for litters produced from acute treated parents.

As shown in Table (5), total number of scrorable bands was altered. They ranged from 15 bands in the negative control to 9 bands after the second week of chronic treatment, to 19 bands after the second week of acute treatment. Hussein and Salam (1985) reported that the protein banding pattern of an organism represents a biochemical genetic fingerprint of that organism and each band reflects a separate transcriptional event. Furthermore, electrophoretic analysis of the protein provides information concerning the structural genes and their regulatory systems that control the biosynthetic pathways of that protein. The changes in the banding pattern in certain progenies might be attributed to three alternatives, i.e. selection, genetic drift and mutational events. These alternatives are mutually exclusive. Elimination of certain alleles due to selection forces needs several generations. Meanwhile, genetic drift acts on several genes and causes a drastic changes in the concerned genotype considering that the disappearance of some alleles which took place in one generation. Therefore, mutational event have to be considered as the reasonable interpretation for the observed changes. Sub fractionation bands could be explained on the basis of the occurrence of gene duplication event, followed by point mutation in one or more of the duplicated genes that encode for particular band. Therefore, two bands will be formed, one of them with the original molecular weight, while the other with the changed one, this conclusion was in accordance with, Abedelsalam *et al.*, (1997).

Table (6): Dominant lethal mutations after treatment of mice with Mach.

	Treatment	Dose (mg/kg)	Time of mating post treatment (days)	No. of males used	Female mated	Overall fertile female [a] % (No. of fertile females)	Implants per female [a] (means ± SE) (No. of females with implants)	Live embryos Per female [a] (means ± SE)	Dead implants per female [a]	Dominant lethal mutation index [b]
	Distilled water (control)	0	0-21	6	12	12	9.2 ±0.328	8.5± 0.31	0.7±0.17	0
chronic	Treated male x normal female	0.0001x 5	1-5	6	12	100(12)	8.3 ±0.33	7.00±0.36	1.25±0.25	16.66
	Normal male x treated female	0.0001x 5	1-5	6	12	100(12)	8±0.30	7.33± 0.25	0.66± 0.14	13.09
	Treated male x treated female	0.0001x 5	1-5	6	12	100(12)	7.66± 0.25	6.22± 0.40	1.44± 0.29	25.95
	Treated male x normal female	0.0001x 10	1-5 & 8-13	6	12	100(12)	7.25± 0.44	5.33± 0.35	1.90± 0.25	52.38
	Normal male x treated female	0.0001x 10	1-5 & 8-13	6	12	100(12)	7.88± 0.32	4.33± 0.22	3.41± 0.37	48.45
	Treated male x treated female	0.0001x 10	1-5 & 8-13	6	12	66.66(8)	6.55± 0.42	3.12± 0.29	3.37± 0.49	62.97
	Treated male x normal female	0.0001x 15	1-5	6	12	75(9)	6.11± 0.38	3.11± 0.51	3.0± 0.33	64.28
	Normal male x treated female	0.0001x 15	8-13	6	12	91.66(11)	7.45 ±0.43	4.22 ± 0.42	3.18 ± 0.46	49.76
	Treated male x treated female	0.0001x 15	1-5 & 8-13& 15-20	6	12	66(8)	5.62± 0.49	2± 0.26	3.62± 0.56	76.19
acute	Treated male x normal female	0.0005	1-5	6	12	100 (12)	7.75±0.35	6.75 ± 0.27	1 ± 0.17	20.23
	Normal male x treated female	0.0005	1-5	6	12	100(12)	7± 0.21	6.6± 0.28	0.33± 0.14	21.42
	Treated male x treated female	0.0005	1-5	6	12	91.66 (11)	6.4 ± 0.41	5.6 ± 0.41	0.81± 0.12	33.33
	Treated male x normal female	0.0005, 0.0005	1-5 & 8-13	6	12	91.66(11)	7.81 ± 0.32	4.18± 0.35	3.36 ± .36	50.71
	Normal male x treated female	0.0005, 0.0005	1-5 & 8-13	6	12	83.33(10)	7.00± 0.33	4.4± 0.42	2.7± 0.49	47.61
	Treated male x treated female	0.0005, 0.0005	1-5 & 8-13	6	12	75 (9)	7.33± 0.33	3.33± 0.38	4.0± 0.52	61.90
	Treated male x normal female	0.0005, 0.0005 0.0005	1-5 & 8-13& 15-20	6	12	83.33 (10)	7± 0.37	2.88± 0.48	3.88± 0.45	65.71
	Normal male x treated female	0.0005, 0.0005 0.0005	1-5 & 8-13& 15-20	6	12	91.66(11)	4.7 ± 0.23	2.9 ± 0.21	1.8 ± 0.29	65.47
	Treated male x treated female	0.0005, 0.0005 0.0005	1-5 & 8-13& 15-20	6	12	75 (9)	6.77± 0.52	2.33± 0.33	4.44± 0.44	72.26

[a] Figures are based on fertile or "pregnant" females ,the respective numbers of which are indicated in parentheses

[b] Dominant lethal mutation index is :

1 - Live implants experiment group per female x 100
Live implants of control group per female

The changes in band intensity or density could be explained on the basis of cytogenetical abnormalities produced by this pesticide. This result is in agreement with those obtained by Abedelsalam *et al.*, (1993) and Hassan (1996), who concluded that the increase in band intensities or densities could be due to gene duplication produced by induction of bridges, breaks and laggards. The disappearance of some bands could be attributed to the loss of some genetic material. Abedelsalam *et al.*, (1993 b) reported that the induction of bridges, breaks, laggards and micronuclei will lead to loss of some of the genetic material.

Dominant lethal mutation assay

In the dominant lethal mutation assay the results showed that a statistically signify-cant decrease in the number of implants / female, compared with that of the negative control, was obtained in treated females with 0.0015 mg/ kg b.w mated with normal males during the third week of mating, while statistically significant reduction in live implants /female was achieved at both doses (acute & chronic) during the second and third weeks of mating, (Table 6). Three way ANOVA completely randomized test shows significant differences between doses while, very highly significant differences between treatments and periods were observed. Singh *et al.*, (2003) evaluated genotoxicity of lomefloxacin, a diflourinated antibacterial drug, employing mouse in vivo dominant lethal mutation assay in germ cells. They found statistically significant decrease in the number of implants / female, compared to control, only in the females mated with males treated with 32 mg/ kg b.w. during the third week of mating , while statistically significant reduction in live implants /female was noticed at both treatments (chronic and acute) during the second and third weeks of mating .

There was also a reduction of fertile mating in treated females mated after treatment of males for second and third week with Match at 0.0010 to 00015 mg /kg. Odeigah, (1997), investigated the effects of formaldehyde exposure in rats and found that the frequency of dominant lethal mutation in female's rats sired by males exposed to formaldehyde was significantly higher than the control group. There was also a reduction of fertile matings in females mated 1-7 days after treatment of males with formaldehyde at 0.125 to 0.500 mg /kg.

In conclusion, the present investigation revealed that the tested insecticide Match was found, at higher doses, to be capable of decreasing cell proliferation in mice bone-marrow. Analysis of mice bone-marrow chromosomes showed that it was proven to be a positive clastogen, since significant different types of aberrations were obtained and a dose-response curve was achieved. Such a conclusion was confirmed by data obtained from the analysis of micronucleated polychromatic erythrocytes. Match was proven to be capable of causing polyploidy, alteration of sperm ultrastructure and alteration of protein banding patterns. Employing dominant lethal assay, mutagenic potentiality of this insecticide was suggested.

REFERENCES

Abdel-Galil A.M. (1996). Effects of the food antioxidant, butylated hydroxyanisole,on foresto-macch and lung tumorigenesis induced in balb/ c mice with benzo (a) pyrene . J. Egypt. Ger. Soc. Zool. 21 (C): 87-97.

Abedelsalam,A.Z, Hassan, H.Z. Soliman, KH. A. and A. Bahieldin (1997). Differential mutagenic activities of two aromatic compounds due to different side chains as revealed by cytological analysis

and biochemical genetic indices Egypt J. Genet. Cytol., 26: 121-142.

Abedelsalam, A.Z, Hassan, H.Z. EL-Domyati, F.M. Eweda, M.A. Bahieldin, A. and Ibrahim, S. A. (1993). Comparative mutagenic effects of some compounds using different eukaryotic systems.. Egypt . J. Genet. Cytol., 22: 129-153

Acharya, U.R., Rathore, R.M. and Mishra M. (2003). Role of vitamin C on lead acetate induced spermatogenesis in Swiss mice. Environmental Toxicology and Pharmacology, 13: 9-14.

Alder, I.D. (1984). Cytogenetic Test in Mammals In: Mutagenicity Testing , a practical approach (Venitt, S.and J.Parry.Eds.), IRL press Oxford.

Anderson, D. (1984). The Dominant Lethal Test in Rodents. In :Mutagenicity Testing : a practical approach (Venitts &J. M. Parry eds). IRL Press. Oxford pp 307-335.

Brusick, D.(1986). Principls of Genetic Toxicology. Plenum Press, New York.

Chauhan, L. K., Pant, N. Gupta, S. K. and Srivastava S.P. (2000). Induction of chromo-some aberrations, micronucleus formation and sperm abnormalities in mouse following carbo-furan exposure. Mutat. Res. 465(1-2): 123-9.

Chhabra, R. S., Herbert, R.A. Bucher, J. R. Travlos, G.S. Johanson J. D. and Hejmancik M.R. (2001) . Toxicology and carcinogenesis studies of p,p'-dichlorodiphenyl sulfone in rats and mice. Toxicol . Sci. 60 (1) : 28-37.

Choudhury, R.C., Mirsa, S. Jagdale, M. B. and A.K. Palo (2002). Induction and transmission of the cytogenetic toxic of 5-fluorouracil in male germline cells of Swiss mice . J. Exp. Clin. Cancer . Res. 21 (2) : 277- 82

Christiansen, T., Korsgaard, B. and Jespersen ,A. (1998). Effects of nonylphenol and 17β-estradiol on vitellogenin synthesis, testicular structure and cytology in male eelpout *Zoarces viviparous*. Journal of Experimental Biology, 201: 179-192.

Cooper, G.H. (1997). The cell: a molecular approach-ASM press. Washington ,Dc.pp.248-9.

Ehling, .U. H., Cumming R. B. and Malling, H.V. (1968). Induction of dominant lethal mutations by alkylating agents in male mice. Mutat. Res., 5, 417- 428.

El –Seedy,A. S., Taha , T. A. Seehy, M. A. and Makhlof A. (2006). Ultrastructure sperm defects in male mice during carcinogenicity of urethane and indoxan. Arab j. Biotech. 9(1): 27-40

Fouad, W.(2003). Biological and biochemical seminal changes in rabbits treated with gossypol. M.Sc. Fac. Agric. Alexandria University.

Hafez, A. M. , El Shehawi, A.M. ,Seedy, M.M. and Seehy M.A. (2004). DNA damage by Decis in human lymphocytes and experimental rodents. Proc. Int. Conf. Genet. Eng. & Appl. April, 8-11: 545-558.

Hart, J.W. and Engberg- Pederson H. (1983). Statistics of the mouse bone- marrow micronucleus test: Counting , distribution and evaluation of results. Mutat. Res. 111:195-207.

Hassan, H. Y. (1996). Evaluation of mutagenic effects of the two insecticides Basudin and Decis on *Vicia faba* plants. Egypt. J. Gent. Cytol., 25(1):27-38

Hussein, E. H. A. and El- Abdin Salam, A. Z. (1985). Evolutionary relationship among *Vicia* species as revealed by electrophoretic studies . Egypt. J. Genet. Cytol. , 14:197-211.

Gollapudi, B. and Kamara O.P. (1979). Application of a simple Gimesa –staining method in the micronucleus test. Mutat. Res. 64:45-6.

Miki K, W. Qu, Goulding, E.H. Willis, W.D. Bunch, D.O. Strader, L.F.

Perreault, S.D. Eddy, E.M. O'Brien, D.A. (2004).Glycera-ldehyde 3-phosphate dehydrogenase-S, a sperm-specific glycol-lytic enzyme, is required for sperm motility and male fertility. Proc Natl Acad Sci U S A. 2004 Nov 23; 101(47):16501-6.

Murthy, R.C., Saxena, D.K. Guptaand, S.K. and Chandra S.V. (1991). Lead induced ultrastructural changes in the testis of rats. Exp. Pathol., 42(4): 95-100.

Reynolds, E.S.(1963). Staining tissue sections for electron microscopy with heavy metals. J. Cell Biol., 17: 203-212.

Odeigah, P. G. (1997). Sperm head abnormalities and dominant lethal effects of formaldehyde in albino rats. Mutat. Res. 17: 389(2-3):141-8.

Saxena, A. K., Dhungel, S. Bhattacharya, S. Jha C. B. and Srivastava A. K. (2004). Effect of chronic low dose of methotrexate on cellular proliferation during spermato-genesis in rats .Arch Androl .50(1):33-5.

Schmid,(1975).

Seehy, M.M. A. (2003). Micro and Macro DNA lesions induced by Decis.M.Sc Thesis, Fac . Agri. Alex. Uni. Alexandria.**Singh,**

A.C., M. Kumar and Jha A. M. (2003). Genotoxicity of lomefloxacin an antibacterial drug in somatic and germ cells of Swiss albino mice in vivo. Mutat. Res. 535(1): 35-42.

Studier, F.W. (1973). Analysis of bacteriophage T7 early RNAs and proteins slab gels. J. Mol. Biol., 79: 237- 248.

Yousef, M.I., K.Bertheussen, M.H. Ibrahim, H.Z. Helmi, S. Seehy M.A. and Salem M.A. (1996).A sensitive sperm – mobility test for the assessment of cytotoxic effects of pesticides .J. Enviro. Science and health .3(1):99-115.

Padmalatha, R. S., Vijayalaxmi ,K. and Tamoxifen K. (2001). Citrate induced sperm shape abnormalities in the *in-vivo* mouse. Mutat. Res. 492(1-2):1-6.

Parry, M.E. and Parry, J. M. (1984). The assay of genotoxicity of chemicals using the budding yeast Saccharomyces cerevisiae. In :Mutagenicity Testing ; a practical approach Cs. Venitt & M. Parry.eds/ IRL Press, Oxford.

Perchec, G., Jeulin, C. Cosson, J. Andre, F. and Billard R. (1995). Relation between sperm ATP content and motility of carp spermatozoa. J. Cell Sci., 108: 747-753.

المستخلص العربى

الضرر الوراثى الصغير والكبير المستحدث بمبيد " ماتش " فى جينوم الفأر

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يهدف البحث الحالى لتقييم ودراسة القدرات المعروفة للمبيد الحشرى " ماتش" والذى يستخدم علي نطاق واسع للسيطرة علي بعض الحشرات التى نصيب الفواكه وذلك للتحقق من احتمالات إحداث أضرار وراثية فى النسيج الجسمى والجنسى للحيوانات الثديية .ولقد تم اختيار الفئران المعملية البيضاء كحيوان ثديى تجريبى لدراسة الأثر الحاد والمزمن عليها وكذا على المجموعة الضابطة بشقيها السالب والموجب (عقار الأندوكسان) – هذا وقد تم توظيف اختبارات السمية التالية:

تحليل كروموسومات خلايا نخاع العظام للشذوذ الكروموسومى المستحدث وتحليل الأنوية الصغيرة فى خلايا الدم غير الناضجة ودراسة التغير فى التركيب الدقيق للحيوان المنوى باستخدام الميكروسكوب الالكترونى ودراسة التفريد الكهربى للبروتين ودراسة التأثير الطفرى للمبيد باختبار السائد المميت ولقد تبين أن المبيد "ماتش" ذو تأثير حاد ومزمن موجب، فقد كانت معدلات المميت السائد بالنسبة لإناث الفئران الملقحة بواسطة الذكور المعاملة أعلى مما شوهد فى المجموعات الضابطة وكان هناك تناقص فى أعداد الإناث المخصبة بواسطة الذكور التى تم معاملتها لمدة 15، 20 يوما على التوالى . وأوضحت نتائج تحليل الشذوذ الكروموسومى والأنوية الصغيرة أن المبيد ذو قدرة تكسيرية موجبة .وباستخدام الميكرسكوب الالكترونى ، أتضح أن المبيد يحدث تغييرات عديدة فى شكل الحيوان المنوى من حيث الحجم وشكل الرأس، وأحداث نوع من الاضطراب النووى، والتكثيف غير الطبيعي للكروماتين بالاضافة الى تأثيره على الغياب الكامل لواحد أو أكثر من ألياف (9+ 2) وغياب بعض الألياف الكثيفة الخارجية .

وأتضح أيضا أنه يؤثر على أنماط شرائط البروتين خاصة الطرز ذات الوزن الجزيئى المرتفع ومن هذا يتضح أن مما يؤكد أن هذا المبيد ذو قدرة طفرية موجبة واضحة .