CYTOTOGENETIC STUDIES ON THE EFFECTS OF ACUTE EXPOSURE TO LANNATE ON MICE

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ABSTRACT

Although carbamate pesticides are widely used, research has shown that they have various side effects. The aim of this study was therefore to investigate the cytogenetic effects of Lannate on mice. Another aim of the study was to investigate the protective effect of olive oil against the cytogenetic effects of Lannate. 36 Swiss albino mice were exposed to various concentrations of Lannate, Lannate and olive oil or were kept as controls. Animals were sampled at two different times (24 and 48 hrs). Lannate increased the number of structural and numerical chromosomal aberrations per cell. On contrary, Lannate produced no effects on the rate of cell division (mitotic index) at either 24 or 48 hrs. Moreover, the use of olive oil gave promising results against Lannate toxicity as it significantly decreased the frequency of chromosomal aberrations.

INTRODUCTION

Carbamates are a member of large group of synthetic pesticides that have been developed and used on a large scale over the last 50 years. Several reports showed that some of these carbamates have many side effects including genetic damage and mutagenic effects **(Moucshen- Dahmen et al., 1984)**. Methomyl is one of the most toxic methyl carbamate pesticides. It is a derivative of carbamic acid that has been widely marketed since 1967 under the trade name (Lannate). Despite its wide application, Lannate is classified by the Environmental Protection Agency (EPA) as a restricted use pesticide (RUP) or a Highly Hazardous class **(Farre et al., 2002)**. The genotoxicity of Lannate has been described by several studies. Some studies showed that Lannate has genotoxic effects including chromosomal aberration and sister chromatid exchanges **(Hemvathy and Krishnamurthy 1987; Quintana et al., 1993; Amer et al., 1996 and Blevins et al., 1997)**. Lannate has been shown to have mutagenic action **(Dean Blevins et al., 1977; Hayes, 1982; Waters et al., 1982 and Wang et al., 1998)**. Lannate has also been demonstrated to have inhibitory effects demonstrated by law mitotic index **(Quintana et al., 1993)**. Genotoxic activity of Lannate may be due to the inhibition of some essential enzymes leading to DNA damage **(Rannug and Rannug, 1984)**, alkalyting activity **(Quintana et al., 1993)** and formation of reactive oxygen species. On Other hand,

other studies showed that Lannate has no genotoxic or mutagenic action **(Wojciechowsk et al., 1982 and Farrow et al., 1984)**. Recent attention has focused on a number of non vitamin antioxidant such as olive oil. Olive oil is a prime component of the Mediterranean diet. It has a protective function and many beneficial effects including the protection against ulcers, gastritis and colon cancer **(Bartoli et al., 2000)**. These beneficial effects of olive oil are thought to be related to its antioxidant and cytopotective effects **(Pompella, 1997)**.

The present study was therefore carried out to investigate the cytogenetic effects of the acute exposure to Lannate on mice and the possible protective effects of olive oil against Lannate toxicity.

MATERIALS AND METHODS

Methomyl was obtained from DuPont Co. U.S.A. as a commercial preparation of "Lannate 90 SP". Olive oil was obtained from Rafael Salgado- Spain (RS).

36 Swiss albino mice (Mus musculus) were obtained from experimental animal farm in Helwan and were used in this study. They were 8-10 weak old and weighted 20-25g at the beginning of the experiment. Pelleted ration and water were offered ad-libitum. Mice were divided into six experimental groups of six animals. Lannate was injected intraperitoneally simultaneously with a single dose of olive oil by gavage as shown in table (1).

All animals were injected intaperitoneally with 1mg/1ml of aquas solution of colchicines two hours before the time of the sacrifice **(Aboul- Ela, 2002)**. Bone marrow preparations for the analysis of chromosome aberrations in metaphase cell were obtained by techniques of **Giri et al., (1986)**. One hundred metaphases per animal were analyzed in order to determine the frequencies of chromosomal aberration. The mitotic index in 3000 cells per group was also analyzed. Statistical analysis was done using one way analysis of variance by SPSS. Mitotic index was analyzed by Chi square analysis by M- state.

RESULTS

I. Chromosomal aberrations:

I.1. Twenty four hours (24 hrs) treatment:

Means ± SE of total aberrant metaphase cells in the control (without any treatment and olive oil group) and treated groups (1/10 LD_{50}, 1/10 LD_{50} of Lannate ± olive oil, 1/5 LD_{50} and 1/5 LD_{50} of Lannate ± olive oil) are present in table (2). The results showed that there was no significant difference between the two control groups (12.00±1.53 and 11.67±1.76 respectively). There was however a significant difference between the treated and the control groups. On the other hand, there was no significant difference between the group treated with 1/10 LD_{50} of Lannate ± olive oil and 1/5 LD_{50} of Lannate ± olive oil (30.33 ± 0.33 and 31.33 ± 0.88 respectively) while there was a significant difference between the group treated with 1/10 LD_{50} of Lannate and 1/10 LD_{50} of Lannate and olive oil (32.67 ± 0.33 and 30.33 ± 0.33 respectively). Moreover, there was a significant difference between the group treated with 1/5 LD_{50} of Lannate and 1/5 LD_{50} of Lannate ± olive oil (35.00 ± 1.45 and 31.33 ± 0.88 respectively). The different types of aberrations

of treated and control groups are presented in table (3) and figures (2-12).

I.2. Forty eight hours (48 hrs) treatment:

Means ± SE of total aberrant cells of the two control groups and the treated groups for 48 hrs are presented in table (4). The result showed that there was a significant difference between the two control groups at one side and the treated groups at the other side. However, no significant difference was observed between two control groups. There was also no significant difference between groups treated with 1/10 LD_{50} of Lannate ± olive oil and groups treated with 1/5 LD_{50} of Lannate ± olive oil (32.67 ± 1.45 and 33.33 ± 0.33 respectively). However, there was a significant difference between the groups treated with 1/10 LD_{50} of Lannate, 1/10 LD_{50} of Lannate ± olive oil (32.67 ± 1.45 and 29.33 ± 0.67 respectively), and between groups treated with 1/5 LD_{50} of Lannate. The 1/5 LD_{50} of Lannate showed the highest mean for aberrant cells (34.67 ± 0.88). The data listed in table (5) illustrate the most prominent type of chromosomal aberrations observed.

II. Mitotic index:

II. 1. Twenty four hours (24 hrs) treatment:

Chi square values of the two control groups and treated groups showed that there were no significant differences between the control and the treated groups (table 6 and 7).

II.2. Forty eight hours (48 hrs) treatment:

Chi square analysis showed that there were no significant differences between the control and the treated groups. These results are presented in table (8 and 9).

DISCUSSION

The results of the acute exposure to Lannate indicated that the acute treatment with Lannate for 24 and 48 hrs caused a significant increase in the aberrations of chromosomes. The results also illustrated that olive oil showed a protective effect and decreased the occurrence of chromosomal aberration. These findings agree with those of **Allen et al., (1982)** regarding ethyl carbamate and related metabolite vinyl carbamate both in vivo and in vitro. **Allen et al., (1982)** found that ethyl carbamate caused an inerease in single chromatid exchanges (SCEs) in vivo only. On the other hand, vinyl carbamate induced SCEs in vivo and in vitro. Similar results were obtained by DeBuyst and **Vanlarebeke, (1982)** who showed that Lannate induced sister chromatid exchanges in human lymphocytes cultures. Also **WHO, (1986)** obtained results agree with the present results on Chinese hamster ovary cells treated with benomyl (a carbamate pesticide) which induced sister chromatid exchanges and chromosomal abnormalities. The results of **Hemavathy and Krishnamurthy, (1987)** who found that Lannate 20 caused chromosomal aberrations on germ cells of mice at 24 hrs agree also with findings of the present study. The results of **Soderpalm-Bernde and Onflet, (1988)** are also in accordance with the reported results on carbaryl in mammalian cells. The authors found that carbaryl induced chromosomal aberrations mainly aneuploidy through the disturbance of spindle fibre. **Ashry, (1990)** studied the acute genotoxic effect of Temik and Carbofuran on bone marrow of rats and found

that Temik and Carbofuran induced numerical and structural chromosomal aberrations such as polyploidy, ring chromosome, end to end dissociation, stickiness, hypoploidy and centromeric attenuation. The results are also in accordance with the finding of **Quintana et al., (1993)** who reported that Lannate induced chromatid aberration frequencies (fragment and bridges) at four hrs in Vicia Faba.

The present results also agree with the result of **Amer et al., (1996)** who reported that Lannate caused maximum chromosomal aberration at 24 hrs after injection intraperitoneally in mice and with those of **Kevekordes et al., (1996)** who noticed that aldicarb (carbamate pesticides) induced increases in the frequency of sister chromatid exchanges in cultured human lymphocytes at 24 hrs. The results of this work are also in an agreement with those of **Topakata et al., (1996)** who found that marshal (carbamate pesticides) induced chromosomal abnormalities in bone marrow cells of rats. On contrary, these results disagree with those obtained by **Waters et al., (1982)** who reported that Lannate was not observed to induce mutation in Drosophila melanogaster. This discrepancy between the results may be attributed to species variation and differences in experimental design. **Manna et al., (2002)** reported that extra virgin olive oil had a protective effect against the cytotoxic effects of reactive oxygen species in human erythrocytes and oxidative damages. Similarly, **Evangelista et al., (2004)** showed that olive and extra virgin olive decreased the chromosomal aberrations and abnormal metaphases induced by acute exposure to anti neoplastic drug cisplatin.

From the previous results it could be reported that Lannate and/or olive oil had no effects on mitotic index in the acute exposure treatment. These results agree with those of **Farrow et al., (1984)** who found that Lannate had no effects on mitotic index in rats exposed to 2, 6, 20 mg/kg B.wt of Lannate for 6, 24 and 48 hrs. On contrary, these results disagree with the results of **Ashry, (1990)** who reported that Temik and Carbofuran decreased the percentage of cell under going mitosis and **Giri et al., (1993)** who showed that carbosulfan induced a cell cycle delay. Similarly, **Quintana et al., (1993)** recorded that Lannate had an inhibitory effect upon cell division demonstrated by low mitotic index in Vicia Faba root at 4 hrs. These differences between the results may be due to differences between types of cells

Table (1): The experimental design of the acute exposure to Lannate (24 and 48h).

Group	Treatment	Dose	Time of exposure/hours	No. of animals.
1	Control	-	24 and 48	6
2	Olive oil	10 mg/ kg B.wt	24 and 48	6
3	Lannate	1/10 LD_{50}	24 and 48	6
4	Lannate and olive oil	1/10 LD_{50} and 10ml/kg .wt.	24 and 48	6
5	Lannate	1/5 LD_{50}	24 and 48	6
6	Lannate and olive oil	1/5 LD_{50} and 10ml/kg.B.wt.	24 and 48	6

Table (2): Means of aberrant cells in animals received Lannate and/or olive oil after 24 hrs.

Group	No. of animals/group	No. of examined cells/animal	Aberrant cells (means ±SE)
Control	3	50	12.00 ± 1.53^{d}
Olive oil	3	50	11.67 ± 1.76^{d}
Lannate 1/10 LD_{50}	3	50	32.67 ± 0.33^{b}
Lannate 1/10 LD_{50}+ olive oil	3	50	30.33 ± 0.33^{c}
Lannate 1/5 LD_{50}	3	50	35.33 ± 1.45^{a}
Lannate 1/5 LD_{50}+ olive oil	3	50	31.33 ± 0.88^{c}

Means having different letters are significantly different at the level of $P < 0.05$.

Table (3): Different types of chromosomal aberrations in animal received Lannate and/or olive oil after 24 hrs.

Groups	Types of aberrations											
	Polyploidy	Hyperploidy	Gap	Fragment	Chromatid deletion	Chromatid break	Centric fusion	Centric attenuation	End to end association	Stickiness	Ring Chromosome	Chromosome break
Control	0.00 ±0.00^{a}	0.00±0.00^{b}	1.00±0.58^{b}	0.33±0.33^{b}	1.00±0.58^{b}	0.33±0.33^{b}	0.33±0.33^{b}	4.67±1.45^{d}	2.00±1.52^{b}	2.33±0.88^{a}	0.67±0.67^{b}	0.00±0.00^{a}
Olive oil	0.33±0.33^{a}	2.33± 0.88^{ab}	0.00±0.00^{b}	1.33±0.88^{b}	0.00±0.00^{b}	1.33±0.88^{b}	1.33±0.88^{b}	3.00±1.15^{d}	4.00±1.15^{b}	4.33±2.02^{a}	0.00±0.00^{b}	0.00±0.00^{a}
Lannate 1/10 LD_{50}	1.67± 0.88^{a}	3.33±0.88^{ab}	6.00±0.58^{a}	2.33±0.33^{b}	6.00±0.58^{a}	2.33±0.33^{b}	2.33±0.33^{b}	9.67±0.88^{b}	4.00±0.58^{b}	13.67±3.18^{a}	1.67±0.33^{ab}	1.00±0.58^{a}
Lannate 1/10LD_{50}+ olive oil	1.00±0.00^{a}	4.00±1.15^{a}	6.00±0.58^{a}	3.33±0.88^{b}	6.00±0.58^{a}	3.33±0.88^{b}	2.33±0.88^{b}	8.00±0.00^{bc}	4.67±0.88^{b}	6.33±0.88^{b}	2.00±0.58^{ab}	0.00±0.00^{a}
Lannate 1/5 LD_{50}	1.00 ±1.15^{a}	1.00±0.58^{ab}	2.00±0.00^{b}	6.00±1.15^{a}	2.00±0.00^{b}	6.00±1. 5^{a}	6.00±1.15^{a}	11.0±0.58^{a}	14.67±2.67^{a}	9.00±1.15^{b}	6.33±2.60^{a}	2.00±1.00^{a}
Lannate 1/5 LD_{50}+ olive oil	0.33± 0.33^{a}	3.33± 0.88^{ab}	5.00±1.15^{a}	6.00±1.15^{a}	5.00±1.15^{a}	6.00±1.15^{a}	6.00±1.15^{a}	7.00±0.58^{c}	2.33±0.33^{b}	11.00±0.58^{ab}	2.67±0.88^{ab}	0.00±0.00^{a}

Means having different letters are significantly different at the level of $p < 0.05$

Table (4): Means of aberrant cells in animals received Lannate and/or olive oil after 48 hrs.

Group	No. of animals/group	No. of examined cells/animal	Aberrant cells (means ±SE)
Control	3	50	12.00± 0.58^{d}
Olive oil	3	50	14.33± 0.67^{d}
1/10 LD_{50} of Lannate	3	50	32.67± 1.45^{b}
1/10 LD_{50} of Lannate+ olive oil	3	50	29.33±0.67^{c}
1/5 LD_{50} of Lannate	3	50	34.67± 0.88^{a}
1/5 LD_{50} of Lannate+ olive oil	3	50	33.33± 0.33^{b}

Means having different letters are significantly different at the level of $P < 0.05$.

Table (5): Different types of chromosomal aberrations in animal received Lannate and/or olive oil after 48 hrs.

Groups	Types of aberrations											
	Polyploidy	Hyperploidy	Gap	Fragment	Chromatid deletion	Chromatid break	Centric fusion	Centric attenuation	End to end association	Stickiness	Ring Chromosome	Chromosome break
Control	0.00±0.00^{b}	0.33±0.33^{c}	1.33±0.88^{c}	2.00±0.00^{c}	3.00±0.00^{d}	0.33±0.33^{b}	2.00±0.00^{b}	5.00±0.00^{d}	2.33±0.88^{ab}	2.00±0.00^{c}	0.33±0.33^{a}	0.00±0.00^{b}
Olive oil	0.33±0.33^{b}	2.33±0.88^{b}	2.33±0.33^{bc}	2.00±0.58^{c}	4.00±0.00^{d}	1.00±0.00^{ab}	3.00±0.58^{b}	3.00±0.58^{c}	3.00±0.00^{ab}	5.33±0.33^{d}	1.00±0.58^{a}	0.00±0.00^{b}
Lannate 1/10 LD_{50}	1.00±0.58^{ab}	0.67±0.33^{bc}	4.67±0.88^{a}	5.67±2.02^{b}	12.67±0.88^{b}	4.00±1.15^{a}	4.00±1.73^{b}	11.67±0.33^{a}	2.00±0.58^{b}	9.67±0.33^{b}	2.67±0.33^{a}	1.33±0.33^{a}
Lannate 1/10 LD_{50}+ olive oil	0.67±0.33^{b}	2.33±0.33^{b}	3.33±0.67^{abc}	4.33±1.45^{bc}	7.33±1.45^{c}	3.33±0.88^{ab}	2.33±0.88^{b}	2.00±0.00^{f} sr	1.33±0.33^{b}	6.33±0.33^{c}	1.33±0.33^{a}	0.00±0.00^{b}
Lannate 1/5 LD_{50}	2.67±0.33^{a}	2.33±0.33^{b}	3.67±0.33^{ab}	9.00±0.58^{a}	14.00±0.00^{a}	4.00±1.15^{a}	7.67±0.88^{a}	8.67±0.88^{b}	4.00±0.00^{a}	11.67±0.88^{a}	3.00±0.58^{a}	0.67±0.33^{ab}
Lannate 1/5 LD_{50}+ olive oil	1.00±0.00^{ab}	4.33±0.33^{a}	5.33±0.33^{a}	3.33±0.88^{bc}	8.00±0.00^{c}	4.33±0.88^{a}	3.33±0.88^{b}	6.00±0.00^{c}	3.00±0.00^{ab}	9.00±0.00^{b}	2.00±1.15^{a}	0.00±0.00^{b}

Means having different letters are significantly different at the level of $p < 0.05$

Table (6): Mitotic index (M.I) in animals received Lannate and /or olive oil after 24 hrs.

Group	Total No. of examined cells	No. of divided cells	No. of non divided cells	M.I
Control	3000	130	2870	4.33
Olive oil	3000	134	2866	4.47
Lannate 1/10 LD_{50}	3000	124	2876	4.13
Lannate 1/10 LD_{50}+ olive oil	3000	126	2874	4.20
Lannate 1/5 LD_{50}	3000	115	2885	3.83
Lannate 1/5 LD_{50}+ olive oil	3000	125	2875	4.17

Table (7): Chi square values of mitotic index in animals received Lannate and /or olive oil after 24 hrs.

Group	*Chi square value*					
	Control	Olive oil	1/10 LD_{50} Of Lannate	1/10 LD_{50} + olive oil	1/5 LD_{50} Of Lannate	1/5 LD_{50} + olive oil
Control						
Olive oil	0.0356					
1/10 LD_{50} Of Lannate	0.103	0.330				
1/10 LD_{50} + olive oil	0.037	0.196	0.0042			
1/5 LD_{50} of Lannate	0.834	1.360	0.278	0.334		
1/5 LD_{50} + olive oil	0.0655	0.258	0.000	0.000	0.351	

Table (8): Mitotic index in animals received Lannate and /or olive oil after 48 hrs.

Group	Total No. of examined cells	No. of divided cells	No. of non divided cells	M.I
Control	3000	132	2868	4.40
Olive oil	3000	128	2872	4.27
1/10 LD_{50} of Lannate	3000	116	2884	3.87
1/10 LD_{50} of Lannate+ olive oil	3000	125	2875	4.17
1/5 LD_{50} of Lannate	3000	111	2889	3.70
1/5 LD_{50} of Lannate+ olive oil	3000	128	2872	4.27

Table (9): Chi square values of mitotic index in animals received Lannate and /or olive oil after 48 hrs.

Group	*Chi square value*					
	Control	Olive oil	1/10 LD_{50} of Lannate	1/10 LD_{50} + olive oil	1/5 LD_{50} of Lannate	1/5 LD_{50} + olive oil
Control						
Olive oil	0.036					
1/10 LD_{50} of Lannate	0.950	0.516				
1/10 LD_{50} + olive oil	0.146	0.0165	0.276			
1/5 LD_{50} Of Lannate	1.720	1.120	0.073	0.745		
1/5 LD_{50} + olive oil	0.036	0.000	0.516	0.0165	1.120	

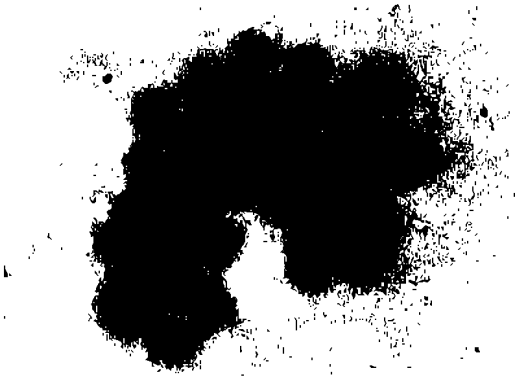

Fig (1): Normal metaphases chromosomes of mice bone marrow cells.

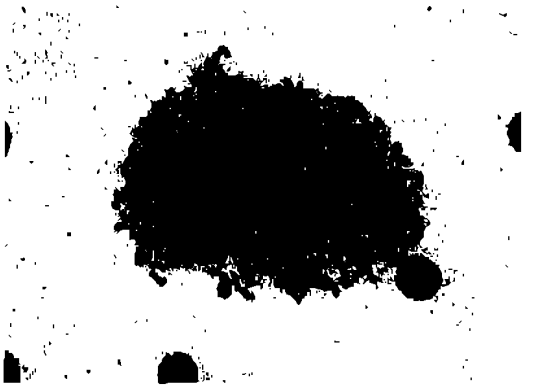

Fig (2): Metaphase chromosomes of mice bone marrow cells after Lannate treatment showing polyploidy.

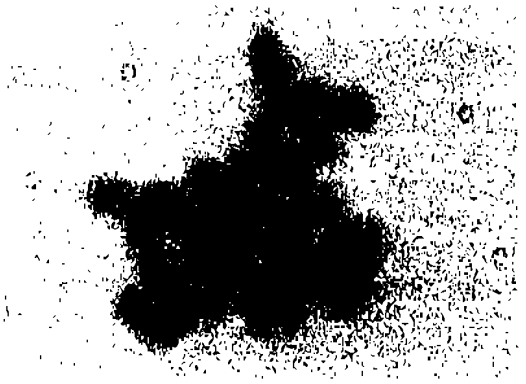

Fig (3): Metaphase chromosomes of mice bone marrow cells after Lannate treatment showing hypoploidy.

Fig (4): Metaphase chromosomes of mice bone marrow cells after Lannate treatment showing (a, b): chromatid break and (c): deletion.

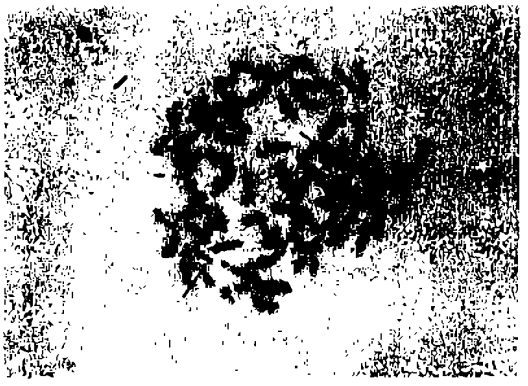

Fig (5): Metaphase chromosomes of mice bone marrow cells after Lannate treatment showing chromosome fragment.

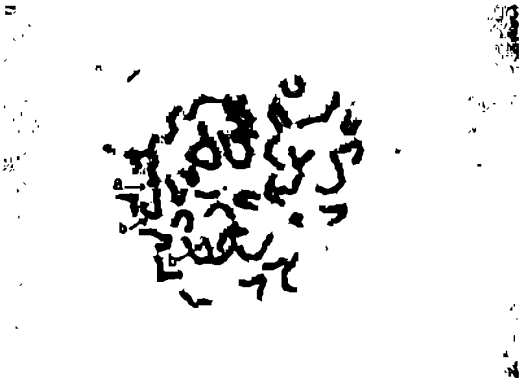

Fig (6): Metaphase chromosomes of mice bone marrow cells after Lannate treatment showing (a): chromosome fragment and (b): deletion.

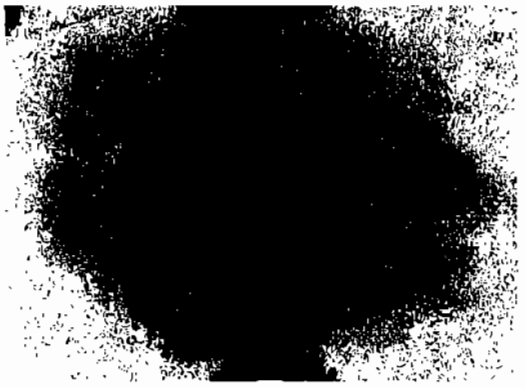

Fig (7): Metaphase chromosomes of mice bone marrow cells after Lannate treatment showing chromosome break.

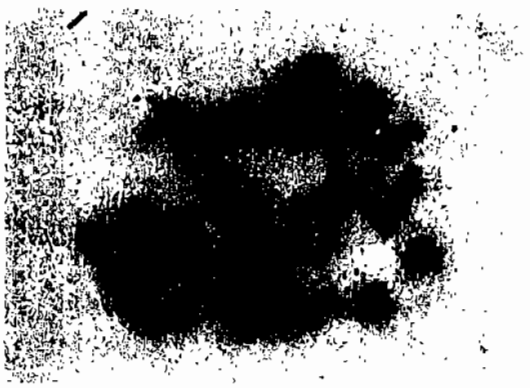

Fig (8): Metaphase chromosomes of mice bone marrow cells after Lannate treatment showing centric fusion translocation.

Fig (9): Metaphase chromosomes of mice bone marrow cells after Lannate treatment showing (a): centromeric attenuation and (b): gap.

Fig (10): Metaphase chromosomes of mice bone marrow cells after Lannate treatment showing stickiness.

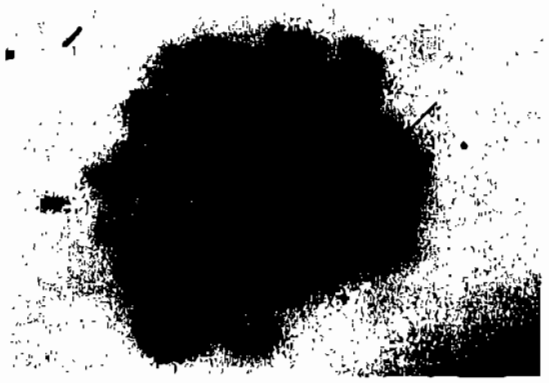

Fig (11): Metaphase chromosomes of mice bone marrow cells after Lannate treatment showing ring chromosome.

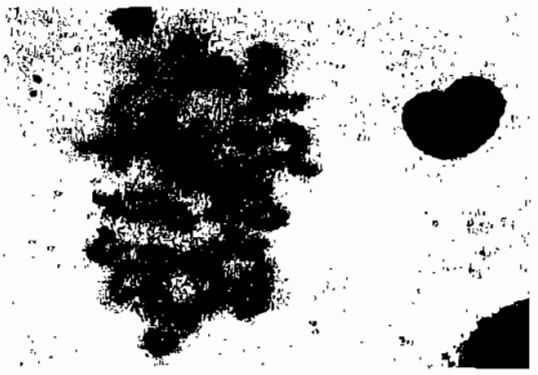

Fig (12): Metaphase chromosomes of mice bone marrow cells after Lannate treatment showing chromosome break.

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الملخص العربى

دراسات وراثية خلوية للتأثير الحاد للاتين على الفئران

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بالرغم من أن المبيدات الحشرية الكاربامينية تستخدم الآن على نطاق واسع إلا أن الأبحاث الحديثة أكدت أن لهذه المبيدات الكاربامينية آثار جانبية، ولهذا أجريت هذه الدراسة لتوضيح التأثير الخلوى السام للاتين على الفئران، وكذلك لتوضيح مدى قدرة زيت الزيتون على حماية الخلية من التأثير السام للاتين، وصممت هذه التجربة من 36 فأر تجارب تعرضوا لتركيزات مختلفة من اللاتين واللاتين مع زيت الزيتون أو إستخدموا كمجموعات ضابطة، وتم أخذ العينات من نخاع الفئران بعد 24 و 48 ساعة. أوضحت النتائج أن اللاتين له قدرة على زيادة التشوهات الكروموسومية العددية والتركيبية فى الخلية، وعلى النقيض أوضحت النتائج أيضاً أن اللاتين ليس له تأثير على معدل الإنقسام الميتوزى لخلايا نخاع عظام الفئران عند 24 و 48 ساعة. وأن إستخدام زيت الزيتون أدى إلى نتائج جيدة ضد التأثير الضار للاتين حيث أنه أدى إلى تقليل معدلات التغيرات الكروموسومية فى الفئران.