GENOTOXIC AND PROBABLE MUTAGENIC EFFECTS OF SOME PESTICIDES ON MICE BONE MARROW CELLS
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ABSTRACT

Lambda-cyhalothrin, Profenofos and Chlorpyrifos are a broad-spectrum pesticides extensively used to control pests for agricultural and household purposes. In the present study an attempt has been made to evaluate its toxicity profile, the cytotoxic, genotoxic and gene mutations effects in-vitro using structural chromosome aberration (SCA) and micronucleus (MN) test systems in erythrocytes assays in mice bone marrow cells. All doses of tested pesticides increased the number of structural chromosomal aberrations and the frequency of micronucleated erythrocytes compared with the control group. While, the results observed that tested pesticides caused a significant increase in the number of structural chromosome aberration and the frequency of micronucleus formation of the metaphase plates of the samples treated with the higher two concentration treatments of 1/10 and 1/40 LD50 of all tested pesticides for 24 hour. In the case of micronucleus test the mice administered for 30, 60, and 90 days, the data revealed satellite associations, chromatid breaks and gaps indicating its effect on chromosomes compared with the control group. The acceptable daily intake (ADI) doses not induce any significant effect. It was also observed that, all tested pesticides induced significant increase in the frequency of chromosome aberration in the bone marrow cells which showed a significant dose-response correlation. Hence, its may be proposed that in-vitro assays like micronucleus and chromosomal aberrations test which indicate genetic damage could be used to study the toxic effect of organophosphorus and pyrethroid pesticides poisoning in humans.

Keywords: Chromosomal aberrations (CA), micronucleus (MN), cytotoxicity, lambda-cyhalothrin (LCT), profenofos, chlorpyrifos, mice bone marrow.

INTRODUCTION

The agricultural chemicals commonly labeled as pesticides are perhaps the largest group of poisonous substances being intentionally disseminated throughout the environment. For some pesticides neither health nor environmental risk evaluations are available. Therefore, at the moment the prevention of occupational and environmental consequences of pesticide use may only be achieved if methodologies and threshold environmental values are developed for the assessment of risk due to handling pesticides. Pre-marketing preventive actions are the primary responsibility of industry and the public health and governmental authorities.

These include discovering the toxicological properties of each pesticide (hazard identification); determine the dose-response relationship [No Observed Effect Level, NOEL, identification], assessing or predicting the exposure level in the various exposure and characterizing the risk. Post-
marketing preventive activities consist of the promotion of proper risk management at the workplace.

This part of the present study aims to evaluate the mutagenic effects of the lambda-cyhalothrin, profenofos and chlorpyrifos, reflected by the production of chromosomal aberrations in maternal bone marrow cells (in vivo) compared with control group.

MATERIALS AND METHODS

Animals: 180 male albino mice were used in this investigation aged 4-5 weeks and of mean weight 20 gram. The animals were randomly housed in appropriate stainless cages in group of 5 animals/cage. The animals were rearranged to 4 classes (1 control + 3 for tested pesticides) and 10 subclasses (1 control + 3 treatment x 3 pesticides) they were also monitored daily for abnormal symptom.

Chemicals: Lambda-cyhalothrin: is a restricted use synthetic pyrethroid insecticide, acute oral LD<sub>50</sub> for rats = 95 mg/kg. b.wt. and (ADI) = 0.005 mg/kg. b.wt. per day. Profenofos: is an organophosphorus insecticide, cholinesterase inhibitor, acute oral LD<sub>50</sub> for rats = 358 mg/kg. b.wt. and (ADI) = 0.01 mg/kg. b.wt. per day. Chlorpyrifos: is organophosphorus insecticide, acute oral LD<sub>50</sub> for rats 150 mg/ kg. b.wt. and (ADI) 0.01 mg/ kg. b.wt. per day.

Animal treatment schedule: Randomized groups of rats housed in cages containing saw dust as bedding and were allocated into 4 groups each group contained 45 males, the first group used as a control while the other groups were treated with tested pesticides at doses of 1/10 LD<sub>50</sub>, 1/40 LD<sub>50</sub> and daily acceptable intake (ADI) through the oral administration for 24 hour. For investigate micronucleus the other groups were treated with tested pesticides at doses 1/10 LD<sub>50</sub>, 1/40 LD<sub>50</sub> and daily acceptable intake (ADI) for 30, 60 and 90 days. Pesticides were given in twice dose weekly through the oral administration.

Sampling:

Chromosomal aberrations test: According to the method described by Alder and El-Tarras (1989):-

Pesticides were injected separately at sublethal level as mentioned above; animals were injected intraperitoneally with a colchicine solution (4 mg/ kg b.wt.) 1-1.5 hour prior to collect tissue sampling.

Animals were killed at 24 hr after treatment. The bone marrow from all animals was transfer to individual centrifuge tubes, and then the cells were centrifuged for 5 minutes at 1000 r.p.m. After centrifugation, the supernatant fluid was discarded completely. Hypotonic solution (kcl 0.56 %) was added slowly, while agitating the tubes to disperse the pellet, and then the tubes were incubated for 17 min. at room temperature.

At the end of hypotonic treatment, the tubes were centrifuged again at 1000 r.p.m. for 5 min. and the supernatant fluids was discarded of freshly prepared cold fixative (methanol + glacial acetic acid 3:1).
After 10 min the cell were centrifuged again and the supernatant was discarded, then the fixation process was repeated. The third fixation step should last 1 hr refrigerate and can be extended to the next day.

**Staining of the slides:** The slides were stained for 30 min., in orcein, the staining was carried out using 2 % orcein in 50 % acetic acid, (2 g. orcein powder were boiled for 1 hr in 100 ml of 50 % acetic acid, filtered when still warm for 30 min.). The stained slides were then transferred to 70 % ethanol for 10 seconds (twice), 90 % for 1 min, and 100 % ethanol for 25 min. After that, the slides were covered with cover slide, left to dry and examined under oil immersion lens.

**Micronucleus test:** The monitoring of micronucleated polychromatic erythrocytes in mice bone marrow were done according to the procedure described by Schmid (1975) with some modifications according to Brusick (1980 b) and Alder (1984).

Staining: The preparations were stained in ordinary vertical staining jar according to method described by Gallapudiand and Kamara (1979).

The slides were fixed in absolute methanol for 5 min., rinsed twice in deionized distilled water staining for 10 min., in Giemsa rinsed again thoroughly in deionized distilled water air-dried cleaned in xylene for 3 min., and mounted.

**Screening of slides:** In this study only polychromatic erythrocytes were scored according to Brusick (1980). Micronuclei were identified as dark-blue staining bodies in the cytoplasm of polychromatic erythrocytes.

**RESULTS AND DISCUSSION**

**Analysis of chromosomal aberrations in rat bone marrow cells.**

Since several studies have shown that, the exposure to pesticides may induce genotoxic effects in occupationally exposed human population. This part of the present study aims to evaluate the mutagenic effects of the lambda-cyhalothrin, profenofos and chlorpyrifos, reflected by the production of chromosomal aberration in maternal bone marrow cells (in vivo) compared with control group, 150 cells were examined and the number of cells with either one or more than one aberration was counted, as well as the structural and numerical aberrations were examined.

Table (1) and Fig (1-12) summarize some chromosomal aberration types that are observed in maternal bone marrow cells after treatment by different doses with tested pesticides. The tested pesticides induced highly significant increase of chromosomal aberration within both high dose compared with the control group and also the data showed dose response relationship that, at high dose 1/10 LD₅₀, the total chromosomal aberration were more than at low dose 1/40 LD₅₀. The results showed the potent mutagenic effect of this pesticides that clear from the data which indicate the significant increase of aberrant cells in high dose, it was mean mutagenic effect of these pesticides only at high dose but low dose (ADI) did not induced any significant effect.
Table (1): Chromatid and Chromosomal aberrations induced by lambda-cyhalothrin, profenofos, and chlorpyrifos at (1/10, 1/40 from LD<sub>50</sub> and ADI) for 24 hours.

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<th>Treatments</th>
<th>Dose (mg/kg b.wt)</th>
<th>Total cells scored</th>
<th>Polyplody</th>
<th>Type of chromosomal aberration</th>
<th>Total % CA cells</th>
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On the other hand the previous mentioned data was disagree with, Bhaskar Gollapudi B., et al. (1995) who noted that cytogenetic abnormalities in mammalian cells both in vitro (rat lymphocyte chromosomal aberration test) and in vivo, and (mouse bone marrow micronucleus test) there was no indication of genotoxic activity for chlorpyrifos in any of these assays. Also, a single i.p. injection of organophosphorus compounds at the highest tolerated dose received by male mice did not produce chromosome damage Noël Degraeve et al., (2002).

Bhunya S. P. and Jena G. B. (2003) stated that a significant induction of chromosome aberrations was observed only after 24 h of exposure with the highest dose (5 mg/kg) of an organophosphate pesticide, monocrotophos.

Also, Ayla Çelik, et al., (2005) stated that cytotoxic and genotoxic effects of lambda-cyhalothrin (LCT) increased the number of the structural chromosomal aberration. Similar results were reported by other investigator, Donbak Y. and Kenan Daglioglu, (2008) who showed that, cyfluthrin increased significantly chromosomal aberration (induce gene mutation).

![Fig. (1): Comparison between the scored chromatid and chromosomal aberrations induced by tested pesticides with control](image1)

![Fig. (2): Comparison between the types of chromatid and chromosome aberrations induced by tested pesticides with control](image2)

![Fig. (3): Chromosomal aberrations in bon-marrow cells after 24 hours as a negative control.](image3)
Fig. (4): Chromosomal aberrations in bone marrow cells induced after treated with lambda-cyhalothrin, at (1/10 LD₅₀) for 24 hours.

Fig. (5): Chromosomal aberrations in bone marrow cells induced after treated with lambda-cyhalothrin, at (1/40 LD₅₀) for 24 hours.

Fig. (6): Chromosomal aberrations in bone marrow cells induced after treated with lambda-cyhalothrin at (ADI) for 24 hours.

Fig. (7): Chromosomal aberrations in bone marrow cells induced after treated with Profenofos at (1/10 LD₅₀) for 24 hours. (X 1000)
Fig. (8): Chromosomal aberrations in bon-marrow cells induced after treated with profenofos at (1/40 LD₅₀) for 24 hours. (X 1000)

Fig. (9): Chromosomal aberrations in bon-marrow cells induced after treated with profenofos at (ADI) for 24 hours. (X 1000)

Fig. (10): Chromosomal aberrations in bon-marrow cells induced after treated with chlorpyrifos at (1/10 LD₅₀) for 24 hours. (X 1000)

Fig. (11): Chromosomal aberration in bon-marrow cells induced after treated with chlorpyrifos at (1/40 LD₅₀) for 24 hours. (X 1000)
Fig. (12): Chromosomal aberrations in bone-marrow cell induced after treated with chlorpyrifos at (ADI) for 24 hours. (X 1000)

Micronucleus test of polychromatic erythrocytes on bone marrow cells:

Tardiff et al., (1994) stated that micronuclei serve as an important endpoint to detect the genetic damage by chemical or radiation in cultured cell and intact organism. Compared to traditional approaches involving the analysis of metaphase chromosomes, micronucleus methods are rapid and easy to learn, and have comparable sensitivity. For these reasons, micronucleus assays are being used with increasing regularity.

In our study the polychromatic erythrocytes micronucleus (PCEM) was scored as the individual erythrocytes containing one, two, three, or more than three micronuclei in the cytoplasm of the cell, and also scored small micronucleus (size of micronucleus less than quarter of the cell) or big micronucleus (size of micronucleus more than quarter of the cell).

The data are presented in Table (2) and illustrated in Fig (13-24) reveal that the pesticide tested induced highly significant increase of (PCEM) within both dose level in comparison with control group and also the data showed dose response relationship that, at high dose 1/10 LD50 the total micronucleate were more than at low dose 1/10 LD50 and (ADI).

The experiments carried out using 1/10 LD50 for 90 days with lambda-cyhalothrin show a total of 47 (PCEM) among 1500 examined cells with a percentage of 3.1 %, while a total of 34 (PCEM) cells were obtained after the treatment with the 1/40 LD50 among 1500 cells with a percentage of 2.3 %, on the other hand (ADI) show a total of 13 PCEM) among 1500 examined cells with a percentage of 0.9 %.

While, the experiments carried out using 1/10 LD50 for 90 days with profenofos show a total of 76 (PCEM) among 1500 examined cells with a percentage of 5.1 %, while a total of 66 (PCEM) cells were obtained after the treatment with the 1/40 LD50 among 1500 cells with a percentage of 4.4 %, on the other hand (ADI) show a total of 15 (PCEM) among 1500 examined cells with a percentage of 1.0 %.

However, the experiments carried out using 1/10 LD50 for 90 days with chlorpyrifos show a total of 92 (PCEM) among 1500 examined cells with a percentage of 6.1 %, while a total of 79 (PCEM) cells were obtained after the treatment with the 1/40 LD50 among 1500 cells with a percentage of 5.3 %, on the other hand the lowest dose show a total of 22 (PCEM) among 1500 examined cells with a percentage of 1.5 %.
Statistical analysis of these results revealed that chlorpyrifos highly significant increase the frequencies of (PCEM) at 1/10 and 1/40 LD<sub>50</sub> doses compared with the control and other tested pesticides, but lambda-cyhalothrin is the lowest one. Generally it could be that all tested pesticides induce significant increase in micronuclei, given evidence that tested pesticides have clastogenic effect.

Table (2): Frequency of mice bone marrow polychromatic erythrocytes micronucleus (PCEM) induced by lambda-cyhalothrin, profenofos and chlorpyrifos at (1/10, 1/40 from LD<sub>50</sub> and (ADI) for 30, 60, and 90 days as respectively.

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<th>Pesticides</th>
<th>Doses</th>
<th>Period</th>
<th>Examined cells</th>
<th>No. of micronuclei</th>
<th>Total No. PCEM cells</th>
<th>% PCEM cells</th>
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The previous mentioned data was agree with data obtained by Stachetti Rodrigues G. et al., (1997) who reported that, chlorpyrifos showed clastogenic potency at doses between 10 and 50 ppm, also showed significant increases in micronuclei frequency, also Titenko-Holland N., et al., (1997) reported that, malathion caused significant increase in micronucleated cells, and Rosadele Cicchetti, et al., (1999) organophosphate phosphamidon induce a dose dependent increase of micronucleated polychromatic erythrocytes.
Fig. (13): Comparison between the scored polychromatic erythrocytes micronucleus (PCEM) induced by tested pesticides with control.

Fig. (14): Comparison between the scored small and big micronucleated (PCEM) induced by tested pesticides with control.

Fig. (15): Photomicrograph of mice bone marrow polychromatic erythrocyte micronucleus (PCEM) as a negative control.

Fig (16): Photomicrograph of mice bone marrow polychromatic erythrocyte micronucleus(PCEM) induced by lambda-cyhalothrin, at (1/10 LD50) for 30 days
Fig (17): Photomicrograph of mice bone marrow polychromatic erythrocyte micronucleus (PCEM) induced by lambda-cyhalothrin, at (1/40 LD$_{50}$) for 60 days

Fig. (18): Photomicrograph of mice bone marrow polychromatic erythrocyte micronucleus (PCEM) induced by lambda-cyhalothrin, at (ADI) for 90 days

Fig (19): Photomicrograph of mice bone marrow polychromatic erythrocyte micronucleus (PCEM) induced by profenofos at (1/10 LD$_{90}$) for 30 days.
Fig. (20): Photomicrograph of mice bone marrow polychromatic erythrocyte micronucleus (PCEM) induced by profenofos at (1/40 LD$_{50}$) for 60 days.

Fig. (21): Photomicrograph of mice bone marrow polychromatic erythrocyte micronucleus (PCEM) induced by profenofos at (ADI) for 90 day.

Fig. (22): Photomicrograph of mice bone marrow polychromatic erythrocyte micronucleus (PCEM) induced after treated with chlorpyrifos (1/10 LD$_{50}$) for 30 days.
Fig. (23): Photomicrograph of mice bone marrow polychromatic erythrocyte micronucleus (PCEM) induced by chlorpyrifos (1/40 LD$_{50}$) for 60 days.

Fig. (24): Photomicrograph of mice bone marrow polychromatic erythrocyte micronucleus (PCEM) induced after treated with Chlorpyrifos at (ADI) for 90 days.

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السمية الوراثية والتأثير الطفيري المحتمل لبعض المبيدات في خلايا نخاع عظام الفئران البيضاء

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غالباً ما ترتبط زيادة استخدام المبيدات في كثير من بلدان العالم بزيادة التلوث البيئي مما يؤدي إلى تغييرات بيئية وصحية خطيرة للناس والحيوان. وتتمثل الخطورة في أن بعض المبيدات الحشرية لها القدرة على أحداث تلف وإضرار بالسامة الوراثية للخلايا الحبيبة بصورة مباشرة أو غير مباشرة.

وقد أجري هذا البحث لدراسة التغيرات الوراثية الخلوية والضرر الخلوي، ودراسة الآثار الضارة على المادة الوراثية الناتجة عن استخدام مبيد حشرى من مجموعة الإيروسيد وهو مبيد الاسماسيلاولين. ومن مبيدات من مجموعة الأمفينوجينات وهم الفئوساس ولهفوس، وكهفوس، وذلك لقياس وقياس قيمة هذه المبيدات الحشرية على أحداث التغيرات الخلوية والتأثير الطفيري المحتمل والتغيرات الكميائية المصاحبة لها في خلايا نخاع العظام وذلك لأهداف أختبارين هما:

1- اختبار التغيرات الكروموسومية التكتيكية والعدوية.
2- اختبار الفردية على أحداث الوراثة الصغيرة.

في خلايا الخمار الغير المضادة

وساهم في دراسة هذا الاختبار 180 فار من ذكور الفئران البيضاء حيث قسمت عشوائياً إلى 4 مجموعات رئيسية متساوية (1 مجموعة كنترول + 3 مجموعات للفئران تحت الاختبار) 45 فار في كل مجموعة. ثم قسمت كل مجموعة رئيسية إلى 3 مجموعات ليكون هناك 10 معاملات ( 1 معاملة كنترول + 3 معاملات لكل مبيد تحت الاختبار) 5 فار في كل
أجريت المعاملة بجربين الفداران عن طريق الفم 10/1، 1/40 (من الجرعة المميزة التoxicية LD50 للمبيدات تحت الاختبار وذلك لمدة 24 ساعة، وقبل انتهاء فترة التعريض بساعتين تم حقن مادة الكولينيس في الغشاء البلطري بتركيز 4 مجم/كلم وذلك في نصف عدد الحيوانات تحت الاختبار. ثم أجراء التشريح والحصول على نخاع العظام لدراسة التغيرات الكروموسومية. أما النصف الآخر من الحيوانات تحت الاختبار تركت للمعالجة عن طريق الفم (من الجرعة المميزة التoxicية LD50 للمبيدات ADI) مرتين أسبوعيا بجرعات 1/10، 1/40، 1/20 يوم وفي نهاية فترة المعاملة تم الحصول على نخاع العظام للدراسة القطرة على أحداث النوويات الصغيرة في خلايا الدم الحمراء غير الناضجة.

وقد أوضحت التحاليل الإحصائية النتائج التالية: أظهر تحليل الشذوذ الكروموسومي على قدرة المبيدات تحت الاختبار في كل الجرعات المستخدمة على أحداث ضرر كبير للمادة الوراثية وظهور عدد من الطرز الكروموسومية الشاذة بصورة معنوية مقارنة بالمجموعة الضابطة، خاصة الفجوة والنظفية، كما أظهر التحليل الاحصائي وجود علاقة خطية موجبة بين التركيز والزيادة في تكرار التغيرات الكروموسومية.

كما أن المبيدات الثلاثة أظهرت تفاوت نسبي في أحداث التأثير الطفري معينا حيث كان مبيد الكلورادينوس أكثر المبيدات أحدثا للتأثير الطفري بليفة مبيد الديموفونوس بينما الاميداديكالوتوريين لم يظهر آثار تأثير طفري طفيف. كما أظهر تحليل النوويات الصغيرة في خلايا الدم الحمراء غير الناضجة قدرة المبيدات على زيادة في تكرار طفرات النوويات الصغيرة بمستويات عالية المعنوية ومرتبطة بالجرعة مقارنة بالمجموعة الضابطة.

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