

Genotoxic Effect of Single and Combined Treatment with Furadan and Sequestrene on the Induction of Micronuclei in Bone Marrow Cells of Male Mice

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Abstract. The genotoxic effects of the carbamate insecticide, Furadan, and Sequestrene each applied singly or combined were studied using the micronucleus test in mice bone marrow cells. Adult male mice were exposed to two doses, the LD50 and half of the LD50 of the two chemicals. The LD50 of Furadan was 0.5mg/kg body weight and the LD50 of sequestrene was 3 mg/kg body weight. All doses induced a significant increase in the number of micronucleated polychromatic erythrocytes (MNPCEs). Moreover, the combined treatment with both chemicals led to stronger effects in the bone marrow of mice represented by higher number of cells with micronuclei compared to the single treatment of each of the two chemicals. The observed results in this study indicated that a single and/or combined treatment of both the insecticide and Sequestrene were clastogenic, however, Furadan was more potent than Sequestrene.

Key Words:

Introduction

Evidence accumulating over the years emphasizing indisputable link between certain insecticides, chromosomal damage and the possibility of gene mutation. There is a wide variety of insecticides, among which carbamates. Furadan is a carbamate insecticide which has been shown to be a potent genotoxic agent in many studies (Nelson *et al.*, 1981; Puszta, 1983; Kar and Singh, 1986; Sharma and Singh, 1990; Sarbhoy *et al.*, 1991; Sabir *et al.*, 1996; and Ahmed *et al.*, 1999). Heavy metals too, have been shown to have genotoxic and clastogenic effects in a variety of organisms. It has been shown, for example, that iron is capable of inducing

chromosome aberrations in mice (Swamy *et al.*, 1993). In *Allium cepa* (Al-Ahmadi, 1994) and in *Aspergillus terreus* (Sabir *et al.*, 1996). The present study was carried out to investigate the possible cytogenetic effects of Furadan and Sequestrene given singly or combined to male albino mice.

Materials and Methods

1. Animals

Male albino mice, three months old and weighting 20-25 g, were used throughout the study as experimental animals.

2. Chemicals:

a. The insecticide (2,3-dihydro-2,3 dimethyl-7 - 6 enzofuranyl methyl carbo-

nate), commercially known as Furadan was supplied by Al-Selouly Agriculture Est., Jeddah, Saudi Arabia.

b. Sequestrene (138 Fe SG 100) which contains 6% Iron in the form of sodium ferric ethylenamin was purchased from the same distributor, Furadan and Sequestrene were dissolved in distilled water.

c. Doses: Two doses of each of the two chemicals were chosen. These two doses were, the LD50 and half of the LD50 for both. The LD50 of Furadan was 0.5 mg/kg body weight (b.w.) and the LD50 of Sequestrene was 3 mg/kg b.w. Half of the LD50 of Furadan was 0.25 mg/kg b.w. and half of the LD50 of Sequestrene was 1.5 mg/kg b.w.

d. Treatment: In the time-course study, mice were injected once intraperitoneally (i.p.) with the two chemicals singly and combined, control animals received i.p. injection of distilled water alone, the applied volume was 0.1 mL/10g b.w.

3. Micronucleus assay:

a. Extraction of bone marrow and preparation of the smears. The preparations were made according to Schmid (1973, 1975) with some modification in fixation and staining based on the method of Heddle *et al.* (1984). The mice were sacrificed by cervical dislocation 24 hours after each exposure and both femora were removed and stripped clean of muscles. The proximal end of each femur was carefully shortened with a pair of scissors to obtain a small opening through which bone marrow cells were aspirated into a centrifuge tube using a syringe containing 2 ml of foetal calf serum. The cell suspension was centrifuged for 5 minutes at 1000 rpm the supernatant was removed with Pasteur pipette. The cells in the

sediment were carefully mixed using Pasteur pipette. A small drop of the viscous suspension was put on the end of a slide and spread by pulling the material behind a cover glass held at angle of 45 degrees. The smears were then air dried.

b. Fixation and staining: The smears were then fixed in absolute methanol for 5 min, and stained for 20 min, and stained for 20 min in a 5% solution of Giemsa in 0.01 M phosphate buffer adjusted to pH 6.8 and then mounted in DPX.

c. Scoring: The slides were coded and examined under the microscope at a magnification of 1250 X. One thousand polychromatic erythrocytes PCEs per mouse were scored for the presence of micronuclei. Micronuclei were identified as dark staining rounded bodies in the cytoplasm of PCEs. Precautions, with regard to scoring and artifacts, were taken as described by Adler (1984) and Sharaf (1992).

d. Statistical analysis: The differences among groups in the incidence of micronuclei in polychromatic erythrocytes were analyzed statistically using Kruskal-Wallis K-sample test (Sokal and Rohlf, 1969).

Results

Table 1 shows the numbers and percentages of micronucleated PCE in bone marrow cells after treatment of mice with Furadan LD50 and half of the LD50, Sequestrene LD50 and half of the LD50 and the combined treatment with LD50 and half of the LD50 of both. As shown in Table 1 and Fig 1, statistically highly significant differences ($p < 0.01$) were observed between the treated groups and control groups in the mean number of MNPCEs per animal. The groups which

Table 1. Incidence of micronucleated polychromatic erythrocytes (MNPCEs) in bone marrow of male albino Mice after Exposure to Furadan and Sequestrene singly or combined.

Group	Dose mg/kg b.w	No. of animals	No. of scored PCEs	No. of MNPCEs	% of MNPCEs	MNPCEs (Mean \pm SD)
Control	-	8	8000	26	0.33	3.25 \pm 1.98
Sequestrene LD ₅₀	3	8	8000	122	1.53	15.25 \pm 3.62**
Sequestrene halfe of LD ₅₀	1.5	8	8000	117	1.46	14.63 \pm 3.07**
Furadan LD ₅₀	0.5	8	8000	318	3.98	39.75 \pm 2.82**
Furadan halfe of LD ₅₀	0.25	8	8000	226	2.83	28.25 \pm 4.74**
Combined treatment (LD ₅₀)	3+0.5	8	8000	439	5.49	54.88 \pm 6.45**
Combined treatment (halfe of LD ₅₀)	1.5+0.25	8	8000	437	5.46	54.63 \pm 7.69**

** Highly Significant (P<0.01)

showed the maximum increase were those of the combined treatments with LD₅₀ and half of LD₅₀ where the number of micronucleated PCEs reached 439 and 437, respectively. On the other hand, Furadan was found to be more potent than Sequestrene.

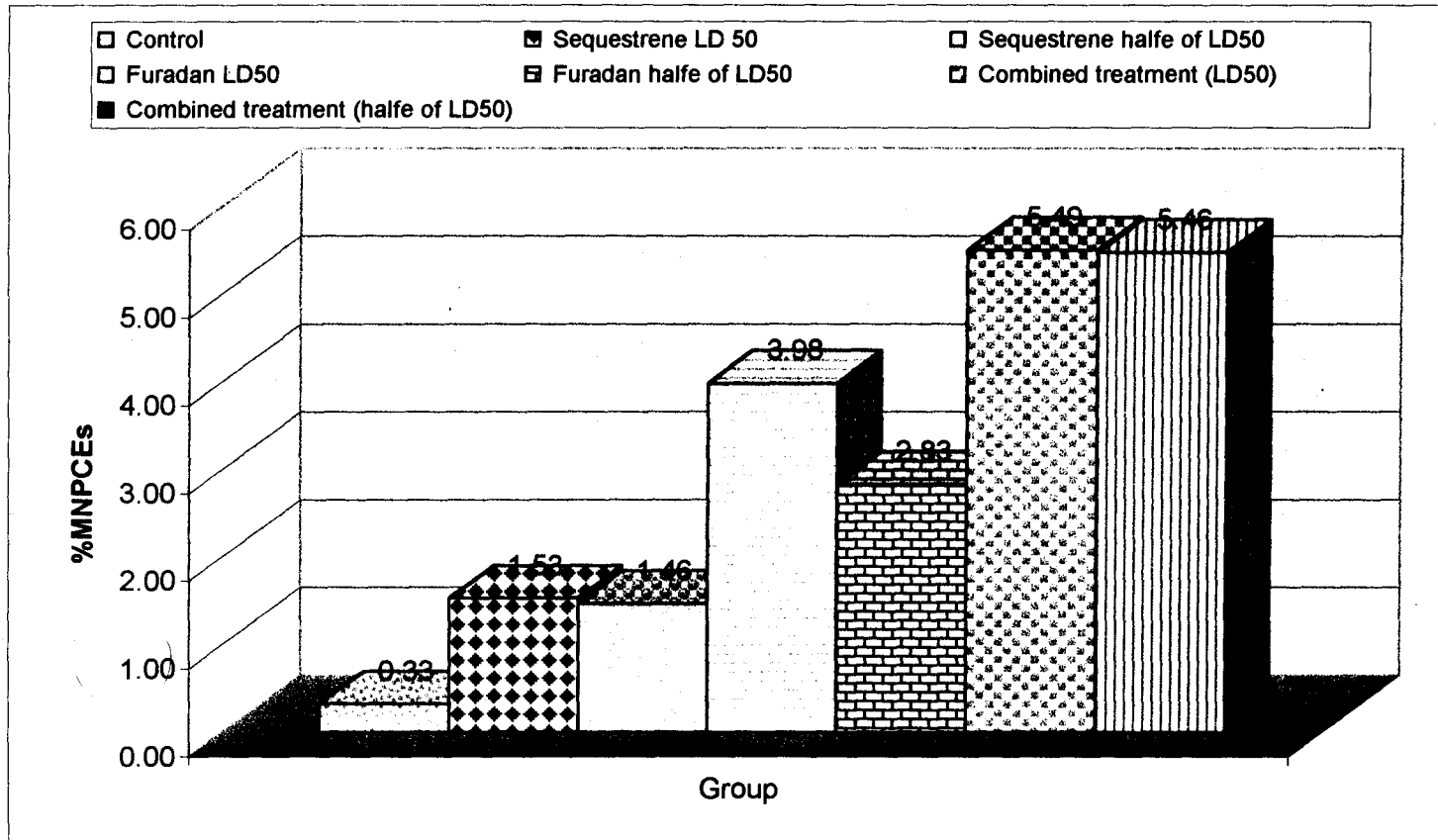
Discussion

The main objective of this study was the identification of the clastogenic effects of Furadan and Sequestrene using the micronucleus test. The most important reason for using this method is that the consequences of chromosome and spindle apparatus damages are visible in the form of nuclear anomalies.

The present study indicates that a single and/or combined treatment of both chemicals induced a significant increase in the number of micronucleated polychromatic erythrocytes (MNPCEs) in bone marrow cells-male mice. The appearance of micronuclei is related either to the loss of chromosome segments as a result of

chromosome breaks or to chromosomal nondisjunction. The positive results of this study are in agreement with Leradi *et al.* (1996) who found that heavy metals (lead, cadmium and zinc) increase the frequency of micronucleated erythrocytes in rodents. In addition, Wei *et al.* (1997) suggested that three carbamate insecticide (propoxur, methomyl and aldicarb) induced a significant increase in micronuclei in Chinese hamster. Moreover, Kumari *et al.* (1997) reported that organophosphorus insecticide (Metasystox-R) induced a significant increase in micronuclei and chromosomal aberrations in Swiss albino mice. The present study also shows that the combined treatment with both Furadan and Sequestrene brought about significantly more genotoxic effects than the single treatment with either of the two chemicals. Such results are in agreement with Al-Ahmadi (1994) who found that the combined treatments of Furadan and Sequestrene led to a decrease in the

Fig. (1) : Effect of different doses of Furadan and Sequestrene administered singly or combined to albino mice .



frequency of mitosis and increase in the rate of chromosomal aberrations in *Allium cepa*. Furthermore, Sabri *et al.* (1996) reported that the combined treatment of the two chemicals (Furadan and Sequestrene) led to an increase in auxotrophic mutants in *Aspergillus terreus*. However the results of the present study disagree with the results obtained by Al-Twaty *et al.* (1999), who found that the combined treatment with both of Furadan and Sequestrene did not produce a significant increase in the number of sex-link recessive lethals in *Drosophila melanogaster*. From the present study, it is possible to conclude that Furadan and Sequestrene have clastogenic effects as they induced a significant increase in the MNPCEs following single and/or combined treatments.

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التأثير السمي الوراثي للمعاملات المفردة والمشاركة للفيورادان والسيكوسترين في استحداث الأنوية الصغيرة في خلايا نخاع العظام لذكور فئران المعمل الصغيرة

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ص ٥٠ ب ٤٢٦١٣ - جده ٢١٥٥١

يهدف هذا البحث إلى دراسة التأثير السمي الوراثي لكل من المبيد الحشري (الفيورادان) والمركب (سيكوسترين) لكل منهما منفرداً أو مشتركين معاً، وذلك باستخدام اختبار تكوين الأنوية الصغيرة في خلايا نخاع العظام لذكور فئران المعمل الصغيرة. وقد تم تعريض ذكور الفئران لجرعتين من المبيد الحشري و المركب كلاً على حدة وأيضاً مشتركين معاً. وهاتان الجرعتان هما الجرعة النصف مميتة ونصف الجرعة النصف مميتة على التوالي وكانت الجرعة النصف مميتة للفيورادان ٠,٥ مجم/كجم وزن الجسم والجرعة النصف مميتة للسيكوسترين ٣ ملجم/كجم وزن الجسم ولقد أدى تعريض الحيوانات لهذه الجرعات من كل منهما منفرداً أو مشتركين معاً إلى زيادة ذات دلالة معنوية في عدد الأنوية الصغيرة في خلايا الدم الحمراء كما أظهرت النتائج المتحصل عليها كذلك زيادة ذات دلالة معنوية في عدد الأنوية الصغيرة بزيادة التركيز من المبيد أو المركب كل منهما منفرداً وأيضاً إلى زيادة عالية عند اشتراكهما معاً، كما اتضح أن الفيورادان له تأثير سمي أعلى من السيكوسترين.