Effect of anticancer drugs on genomic DNA and chromosomes in Ehrlich ascites tumor bearing female mice

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Mona H. Hussein*; Zakaria A. Teleb;Reda A. Mogheib*and Maged I. Ibrahim****

*Department of Genetics, Faculty of Agriculture, Cairo University, Giza, Egypt.

**Molecular Drug Evaluation, National Organization for Drug Control and Research (NODCAR), Giza, Egypt.

ABSTRACT

The antioxidant activity, DNA damage and chromosomal aberrations were studied under the effect of cis-diaminedichloro platinum (CDDP) and vinblastine sulphate (VLB) in Ehrlich ascites tumor bearing female mice. The two doses of CDDP and VLB were found to decrease the hepatic glutahione (GSH) content and total protein. Lipid peroxidation (MDA) increased significantly in the first and second weeks of administration compared to the control. Also, losses in body and liver weights were observed. Genomic DNA concentration was decreased by CDDP and VLB to 15.0%, 32.0%, 22.0% and 43.5%, respectively, DNA banding pattern of liver tissue on agarose gel (1.3%) electrophoresis was found compared to control. Serum protein banding pattern under the effect of the two drugs SDS-PAGE was observed clearly after 2 weeks of administration, but not after the first week. The percentage of total chromosomal numerical aberrations of bone marrow cells were significantly increased for CDDP and VLB to 304.8%, 457.1%, 238.1% and 290.5%, respectively. Also the percentages of total chromosomal structural aberrations of bone marrow cells were significantly increased to 372.7%, 581.8%, 101.0% and 150.0%, respectively in the second week. The effect of these drugs was dose-dependant. It is concluded that these anticancer drugs are harmful and precaution should be considered when administrated in human.

Key words: *Cisplatin, Vinblastine, Ehrlich ascites tumor, Oxidative stress.*

INTRODUCTION

Carcinoma is a type of cancer that represents (80-90% of cases) and originates in epithelial tissue, which includes the skin, the covering, lining of the organs and internal passageways (Murphy *et al.*, 1997). Ehrlich Ascites Tumor (EAT) is a type of tumors orginates from the carcinomas tumors.

Cis-diaminedichloroplatinum (II) CDDP) and Vinblastine Sulphate (VLB) are chemotherapeutic agents. They are widely used in the treatment of different types of malignant tumors, such as testicular cancer, lung cancer and breast carcinoma (De Pas *et al.*, 2001). Cisplatin has a genotoxic effect in germ cells, causing the development of various forms of abnormalities in the sperm heads in mice (Khynriam and Prasad, 2003). Edelweiss *et al.* (1995) investigated the clastogenicity of CDDP on Wistar rat bone marrow cells; the most impressive effect of a single dose of CDDP was an increase in the frequency of chromosomal aberrations and in the number of abnormal metaphases after 24 hr of interapertoneal administration (Choudhury *et al.*, 2000).

The ability of cisplatin to react with nucleophilic bases in DNA to form intra- and interstrand cross-links has been suggested to be the main mechanism behind its anticancer activity (Kartalou and Essigmann, 2001). Cisplatin causes oxidative stress mainly by inceasing lipid peroxidation and depletion of glutathione, which in turn induces apoptosis of renal proximal tubule cells and consequent kidney dysfunction (Zhang and Lindup, 1993 and Chang *et al.*, 2002).

Cisplatin induced hepatotoxicity was enhanced by elevating expression of CYP2E1 and may involve increasing the production of reactive oxygen species (ROS) and oxidative stress (Lu and Cederbaum, 2006). The hepatotoxicity induced by cisplatin has been rarely characterized.

The vinca alkaloid vinblastine is an important antitumor agent which used for the treatment of testicular cancer; vinblastine binds to tubulin subunits and inhibits tubulin polymerization, thus disrupting the dynamic instability of spindle microtubules (Jordan and Wilson, 2004). Choudhury *et al.* (2004) investigated the clastogenic effect of VLB in bone marrow cells of mice and increases the micronuclei (MN) induction.

Vinblastine inhibits cell proliferation in a concentration dependent manner *in vitro* (Jordan *et al.*, 1991) and *in vivo* (Jagetia and Jacob, 1992). Salassidis *et al.* (1992) found an increase in the frequency of micronuclei after vinblastine treatment in mouse liver cells culture. Vinblastine is a potent mitotic inhibitor and reduces cell proliferation (Jordan *et al.*, 1991 and Jagetia and Jacob, 1992).

This study aims to investigate the effect of two doses of CDDP and VLB on the antioxidant activity, DNA and chromosomal changes of bone marrow cells in Ehrlich ascites tumor bearing female mice.

MATERIALS AND METHODS

Chemicals

Cis-diaminedichloro platinum (II) (CDDP) and Vinblastine sulphate (VLB) (KUP under the technical assistance of United Douglas Pharm., USA. Importer: EIMC Pharmaceuticals Co.), thio barbaturic acid (TBA) (Sigma, USA), Ellman's reagent [5,5\dithiobis-(2-nitrobenzoic acid)] (Sigma, USA), 1-chloro 2,4 dinitrobenzene (CDNB) (Fluka, India), ρ-nitrophenyl-phosphate-sodium salt (Sigma, USA) and bovine serum albumin (Sigma, USA), were used.

Animals and tumor maintenance

Inbred female albino mice (*Mus musculus*), weighting 25-30g, 10-12 weeks old were used. Animals were obtained from the animal house of the National Organization for Drug Control and Research (NODCAR), Giza, Egypt; they were kept under environmental and nutritional conditions for 2 weeks.

The tumor was maintained in female Swiss albino mice by weekly interapertoneal (i.p) transplantation of 2.5×10^6 cells in the National Cancer Institute (NCI), Cairo, Egypt. Tumor cells were taken from transplanted animals after 7 days of transplantation and resuspended by appropriate volume of saline, then 2×10^6 cells (approximately 0.2 ml) of this suspension were injected (i.p) in each female mouse.

Experimental design

This study was carried out on 125 female albino mice, each one was injected (i.p) with 2.5×10^6 (0.2 ml) of EACCs, after 7 days of tumor transplantation; animals were divided into 5 groups, each group contained 25 animals.

Group (1): the mice were injected (i.p) with NaCl 0.9% once weekly for two weeks it represented the control.

Group (2): the mice were injected (i.p) with CDDP "3mg/kg" (Choudhury *et al.*, 2000) once weekly for two weeks.
Group (3): the mice were injected (i.p) with CDDP "6mg/kg" once weekly for two weeks.
Group (4): the mice were injected (i.p) with and VLB "0.9mg/kg" (Satya-Prakash *et al.*, 1986) once weekly for two weeks.
Group (5): mice were injected (i.p) with VLB "1.8mg/kg" once weekly for two weeks.

After 24 hours of the drugs administration in the first and second week, blood was collected to separate serum protein patterns on SDS-PAGE. Body and liver tissues were weighted and liver homogenate was prepared for different determinations.

Preparation of tissue liver homogenate

Animals were killed by decapitation and bleeding. Liver was rapidly removed and weighed and homogenized in Tris-HCl (0.1 M, pH 7.4) using an electric homogenizer to prepare 10% w/v (0.5 g/5 ml) homogenate. The homogenate was centrifuged at 5000 rpm for 15 min at 4°C and the supernatant was used for determining the different parameters (Orafidiya *et al.*, 2004).

Determination of total protein

Total protein was determined in liver homogenate according to Lowry *et al.* (1951).

Determination of lipid peroxidation (MDA) concentration

Lipid peroxides formation was determined in liver homogenate using TBARS according to the method of Buege and Aust (1978).

Determination of glutathione reduced (GSH) content

Glutathione reduced was determined in liver homogenate according to the method of Ellman (1979) with some modifications (Ahmed *et al.*, 1991).

Extraction and purification of genomic DNA

Genomic DNA was isolated from the mouse liver tissue according to Surzycki (2000). Genomic DNA was fractioned on agarose gel electrophoresis (1.3%) and ethidium bromide staining.

Cytogenetic analysis

The Cytogenetic analysis of chromosomes of bone marrow cells was carried out according to Nichols *et al.* (1972). Mice were injected 0.1ml of colchicine (0.5%) after 1-1.5 hr, they were killed. The chromosomes were prepared and stained by Giemsa stain using phosphate buffer (pH 6.8).

Statistical analysis

Data were subjected to statistical analysis as mean±S.D according to Fisher and Yates (1957); Snedecor and Cochram (1967) and practicing statistical analysis of Student's *t*-test. Differences were regarded as insignificant at $P>0.05$, significant at $P>0.025$, highly significant at $P>0.0025$ and very highly significant at $P<0.0005$.

RESULTS AND DISCUSSION

The results illustrated the genotoxicity and the toxic effect of the two anticancer drugs cispaltin (CDDP) (3&6mg/kg b.w) and vinblastine sulphate (VLB) (0.9&1.8mg/kg b.w) in Ehrlich ascites tumor bearing female mice.

Effect of CDDP and VLB on body, liver weights and their ratio in Ehrlich ascites tumor bearing female mice in the second week

Data in Table (1) showed that the two doses of CDDP and VLB exerted a highly significant decrease in body and liver weights, while the high dose of VLB on liver/body weight appeared to be less effective. The ratio of liver/body weights appeared to be high

(28.57%) under the effect of CDDP (3mg/kg b.w), while the high dose of VLB (1.8 mg/kg b.w) gave a lesser ratio (9.5%). Teranishi *et al.* (2001) showed that the decrease in body weight of CDDP-treated animals is ameliorated by the combined administration of α-tochopherol. This might reflect an improvement of the general condition including kidney and liver functions. These results are in agreement with Pratibha *et al.* (2006); they found that the long-term treatment of CDDP (0.4mg/kg i.p.) for 8 weeks caused a statistical significant decrease in the body and organs of rats. It was found that the body weight loss is associated with CDDP nephrotoxicity (Sekine *et al.*, 2007).

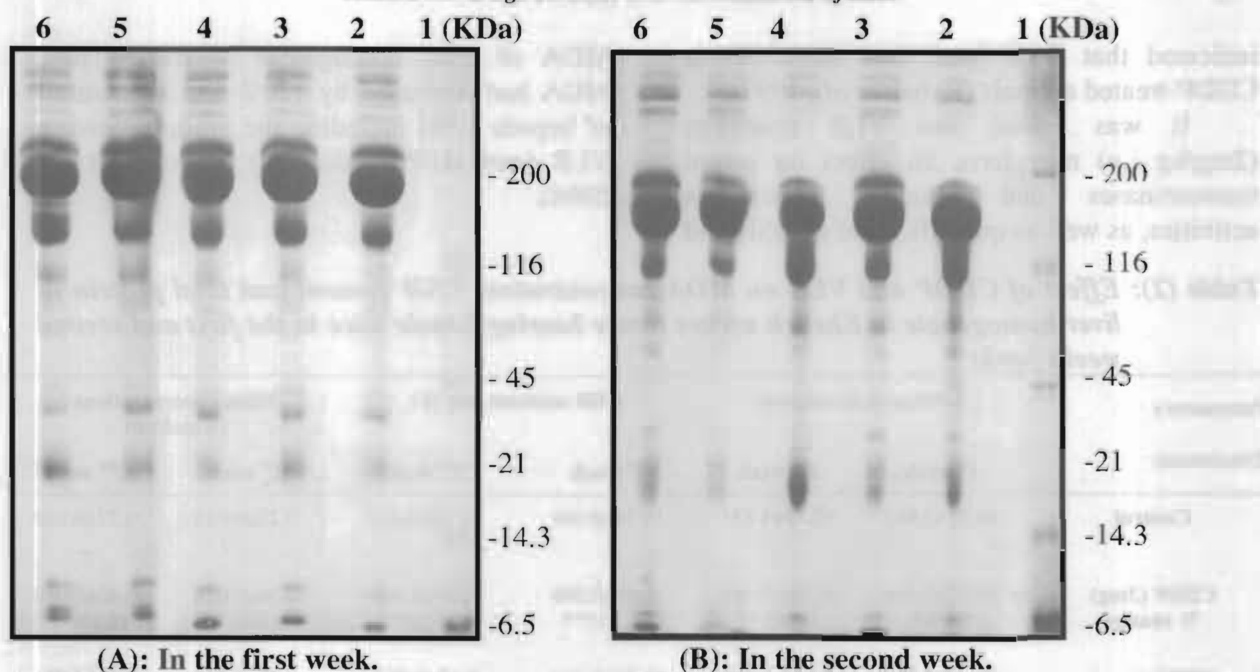
Table (1): Effect of CDDP and VLB on body, liver weights and their ratio in Ehrlich ascites tumor bearing female mice in the second week (n=8).

Treatments Parameters	Body weight (g) (b.w)	Liver weight (g)	Ratio of Liver /b.w
Control	31.20±1.3	1.3±0.25	0.042
	-	-	-
CDDP (3mg)	20.40±1.14	1.1±0.07	0.054
% change	34.62%***	15.4%†	28.57%
CDDP (6mg)	19.30±0.05	0.9±0.09	0.047
% change	38.14%***	30.77%†	11.90%
VLB (0.9mg)	24.10±1.9	1.2±0.05	0.050
% change	22.76%**	7.7%†	19.05%
VLB (1.8mg)	23.90±0.9	0.9±0.1	0.038
% change	23.40%***	30.77%†	9.52%

The % changes were calculated from control.

Effect of CDDP and VLB on serum protein patterns by SDS-PAGE in Ehrlich ascites tumor bearing female mice in the first and second weeks

The results in Fig. (1) showed that the serum protein patterns exerted variability in molecular size between 6.5-200 KDa; the most intensive bands were observed about 116-200 KDa, then decreased to 21-45 KDa. Also, there are some changes in serum protein banding patterns between groups in the first and second week, where the protein profiles have changed drastically in the second week under the effect of these drugs. It was found a statistically insignificant decrease in protein levels was recorded after CDDP treatment (0.4mg/kg i.p.) (Pratibha *et al.*, 2006).



(A): In the first week. **(B): In the second week.**

Fig. (1): Serum protein profiles (A) in the first week, (B) in the second week by SDS- PAGE (12.5%). (1) Marker (6.5-200 KDa), (2) Control, (3) CDDP (3mg), (4) CDDP (6mg), (5) VLB (0.9mg) and (6) VLB (1.8mg).

Effect of CDDP and VLB on MDA concentration, GSH content and total proteins of liver homogenate in Ehrlich ascites tumor bearing female mice in the first and second weeks

As shown in Table (2); the treatments with CDDP and VLB revealed high percentages of MDA. The high concentration of each drug exerted a high percentage of MDA (TBARS) concentration; this is due to the increase in lipid peroxidation. The effect was dose-dependent. Reports from Pratibha *et al.* (2006) indicated that CDDP increased MDA in treated rats.

Glutathione reduced content was reduced under the effect of the two anticancer drugs by the two concentrations in either the 1st week or 2nd week as compared to the control. The concentration of GSH was time and dose-dependent. Also as shown in Table (2) and Fig. (3), the total proteins had slightly inhibited as compared to the control. This reduction had altered according to the drug dosage and the duration time.

It was shown that the uptake of cisplatin could inhibit protein synthesis, deplete reduced glutathione, and damage mitochondria (Kuhlmann *et al.*, 1997). Cellular GSH content decreased after the treatment with cisplatin alone, may hint the possibility of a less conjugation of cisplatin with GSH and availability of more drug to bind with DNA and causing genotoxic effects (Khynriam and Prasad, 2003).

CDDP may be closely associated with glutathione metabolism since GSH is responsible for the detoxification of active CDDP hydrolysates (Bier, 1990). CDDP reacts with sulfhydryl groups including glutathione and the methionine groups in proteins (Lempers and Reedifk, 1990). These results

indicated that GSH level had decreased in CDDP-treated animals (Rybak *et al.*, 1997).

It was found that VLB treatment (2mg/kg i.v) may have an effect on serum transaminases and alkaline phosphates activities, as well as quantification of GSH and MDA of liver homogenate of treated rats, MDA had increased by 120% and a downfall of hepatic GSH including the group receiving VLB (until 210% reduction) (Lahouel *et al.*, 2004).

Table (2): Effect of CDDP and VLB on MDA concentration, GSH content and total protein in liver homogenate in Ehrlich ascites tumor bearing female mice in the first and second weeks (n=8)

Parameters / Treatments	Total protein (mg/ml)		GSH contents (mg %)		MDA concentrations (n mol/ml)	
	1st week	2nd week	1st week	2nd week	1st week	2nd week
Control	96.38±2.892	92.72±1.854	19.54±0.391	14.76±0.422	0.23±0.012	0.27±0.010
	–	–	–	–	–	–
CDDP (3mg)	87.78±2.634	82.70±2.482	16.96±0.346	7.34±0.364	0.36±0.018	0.40±0.018
% change	8.9%*	10.8%**	13.2%***	50.3%***	6.5%***	48.2%***
CDDP (6mg)	90.18±2.706	81.56±1.632	17.10±0.364	6.12±0.394	0.37±0.019	0.43±0.014
% change	6.4%†	12.0%***	12.5%***	58.5%***	60.9%***	59.3%***
VLB (0.9mg)	86.54±2.596	82.98±2.490	11.81±0.421	8.05±0.356	0.30±0.015	0.33±0.015
% change	10.2%*	10.5%**	39.6%***	45.5%***	30.4%**	22.2%**
VLB (1.8mg)	93.30±2.802	81.2±2.436	18.02±0.362	7.17±0.384	0.32±0.016	0.36±0.016
% change	3.2%†	12.4%***	7.8%**	51.4%***	39.1%***	40.7%***

The % changes were calculated from control.

Effect of CDDP and VLB on genomic DNA in Ehrlich ascites tumor bearing female mice in the second week

Table (3) shows the purity of genomic DNA of liver was 1.6-2.0. Genomic DNA concentration had decreased by CDDP (3&6mg/kg) and VLB (0.9&1.8mg/kg) to 15.0%, 32.0%, 22.0% and 43.5%, respectively. This finding indicates that the effect was dose-dependant.

Figure (4) illustrates the different lanes profiling the genomic DNA on agarose gel (1.3%). As revealed from the figure, the molecular weights of all genomic DNA isolated from liver tissue in the second week appeared to be more than 2.5Kbp. Genomic DNA of control group showed definite bands, while the other bands of different groups gave damaged and smear bands. This may be due to the cytotoxicity of CDDP and VLB on genomic DNA. As indicated by Cemazar *et al.* (2002), the major target of CDDP is DNA; an increased amount of platinum in the cells, which consequently results in increasing amount of platinum bound to DNA, may result in increasing cytotoxicity of CDDP by a reduced number of Ehrlich ascites tumor cells in female mice after 24 hours of treatment. Beata *et al.* (2005) found that the oxidative stress and cellular biomolecules damage (lipids, proteins and DNA) were induced by platinum compounds, this could be due to the formation of hydrogen peroxide and hydroxyl free radicals in the cell which can cause DNA damage (Ramadan *et al.*, 2001).

It was shown that the alkylating agents exert their action through DNA cross-linking or by inducing oxidative stress (Hasinoff *et al.*, 2005). It was pointed out that the chemotherapeutic agents used in the treatment of neoplasia are either clastogenic or mutagenic; these agents mainly induce damage to the DNA in the form of primary DNA adducts or secondary lesions that may lead to chromosomal aberrations and point mutations (Jagetia and Jacob, 1992).

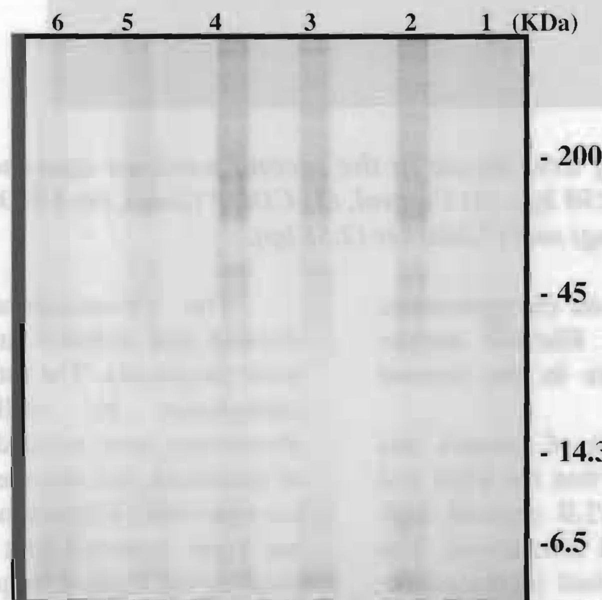


Fig. (2): liver proteins profilesn by SDS-PAGE (12.5%). (1) Marker (6.5-200 KDa), (2) Control, (3) CDDP (3mg), (4) CDDP (6mg), (5) VLB (0.9mg) and (6) VLB (1.8 mg).

Table (3): Effect of CDDP and VLB on genomic DNA of liver tissue in Ehrlich ascites tumor bearing female mice in the second week (n=8).

Parameters / Treatments	DNA purity and concentration	
	Purity (Abs_{260}/Abs_{280})	Total DNA concentration (ug/ml)
Control (-V)	2.06	75.25±1.30
CDDP (3mg)	1.64	64.00±2.50 15.0%**
CDDP (6mg)	1.74	51.20±2.60 32.00%***
VLB (0.9mg)	1.96	58.75±3.80 22.00%**
VLB (1.8mg)	1.77	42.50±1.50 43.50%***

The % changes were calculated from control.

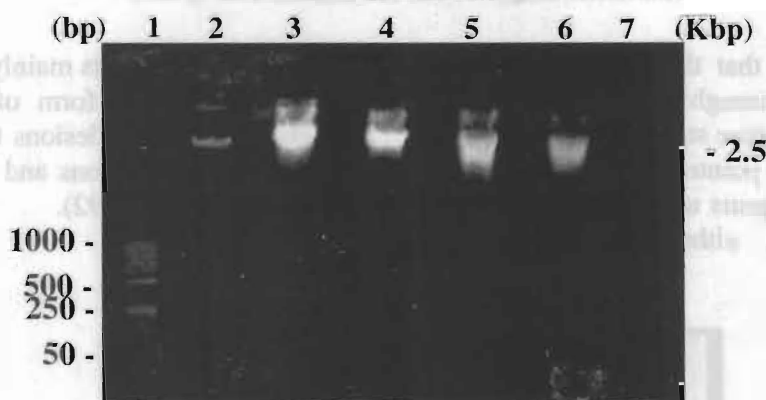


Fig. (3): Genomic DNA of liver tissue in the second week on agarose gel electrophoresis 1.3%), (1) Marker (50 bp), (2) Control, (3) CDDP (3mg), (4) CDDP (6mg), (5) VLB (0.9 mg), (6) VLB (1.8mg) and (7)Marker (2.5Kbp).

Effect of CDDP and VLB on chromosomes of bone marrow cells in Ehrlich ascites tumor bearing female mice in the second week.

Tables (4&5), Fig. (4) of control and Figs. (5, 6, 7&8) revealed that the high and low doses of CDDP and VLB exerted high frequencies of chromosomal aberrations. The total numerical aberrations had increased for CDDP (3&6mg/mg b.w) to 304.8% and 457.1% respectively, while for VLB (0.9&1.8mg/kg b.w), the aberrations had increased to 238.1% 290.5% respectively as compared to control. Also, the mitotic index (MI) decreased for CDDP (3&6mg/mg b.w) to 3.5% and 3.1% respectively, while for VLB (0.9&1.8mg/kg b.w) it decreased to 5.1% and 4.8% respectively as compared to control (Table 5).

In addition, the total structural aberrations have increased for CDDP (3&6mg/mg b.w) to 372.7% and 581.8%, respectively, while for VLB (0.9&1.8mg/kg b.w) the aberrations had increased to 101.0% and 150.0% respectively as compared to control (Table 6).

The chromosomal aberration pattern showed that deletion and fragments occurred more frequently. The total numbers of aberrant metaphases as well as chromosomal aberrations were noticed to be highest at 24 hr of treatment, but decreased appreciably during later periods (Khynriam and Prasad, 2003). It has been reported that chemicals in general produce the highest frequency of aberrations in rodents 24 hr after single exposure (Giri *et al.*, 1998).

It was found that cisplatin treatment to lymphoma tumor-bearing mice caused the development of chromosomal aberrations in bone marrow cells as well as in Dalton's lymphoma cells (Giri *et al.*, 1998). Choudhury *et al.* (2004) reported that VLB has a clastogenic effect and increased the micronuclei (MN) induction in bone marrow cells of mice. Genotoxicity studies of the CDDP and VLB as anticancer drugs were carried out in Ehrlich Ascites tumor bearing mice using chromosomal aberrations (CAs) and other parameters of oxidative stress for the genotoxic effect (Siddique and Afzal, 2004).

Table (4): Effect of CDDP and VLB on chromosomes number of bone marrow cells in Ehrlich ascites tumor bearing female mice in the second week (n= 8).

Types of chromos. aberrations / Treatments	MI %	Numerical aberrations: Endomitosis	Numerical aberrations: Poly ploidy	Total aberrations
Control	5.9%	0.8±0.33 -	1.3±0.42 -	2.1±0.18 -
CDDP (3mg) **% change**	↓3.5%	4.7±0.21 487.5%***	3.8±0.30 192.3%***	8.5±0.73 304.8%***
CDDP (6mg) **% change**	↓3.1%	7.1±0.33 787.5%***	4.6±0.26 253.9%***	11.7±1.00 457.1%***
VLB (0.9mg) **% change**	↓5.1%	3.9±0.11 387.5%***	3.2±0.15 46.2%***	7.1±0.61 238.1%***
VLB (1.8mg) **% change**	↓4.8%	4.4±0.21 450.0%***	3.8±0.25 92.3%***	8.2±0.71 290.5%***

The % changes were calculated from control.

Table (5): Effect of CDDP and VLB on chromosomes structure of bonemarrow cells in Ehrlich ascites tumor bearing female mice in the second week (n= 8).

Types of chromos. aberrations / Treatments	Deletion	Cent. atten.	Cent fus.	Ring chrom.	End to end assoc.	Frag.	Gap	Total aberrant.
	Structural aberrations							
Control	2.1±0.23 -	1.9±0.19 -	1.5±0.24 -	1.1±0.12 -	1.2±0.11 -	2.0±0.18 -	1.2±0.15 -	11±0.95 -
CDDP (3mg) **% change**	11.3±1.03 438.1%***	6.1 ±0.45 273.7%***	5.0±0.56 233.3%***	2.6±0.40 136.4%***	4.3±0.33 258.3%***	15.3±1.19 665.0%***	4.1±0.32 241.7%***	48.7±4.21 372.7%***
CDDP (6mg) **% change**	18.8±0.67 795.2%***	9.0±0.64 375.7%***	6.3±0.36 320.0%***	3.5±0.20 218.2%***	5.5±0.31 358.3%***	25.0±0.60 1250.0%***	6.9±0.66 475.0%***	75.0±6.48 581.8%***
VLB (0.9mg) **% change**	4.2±0.40 100.0%***	4.1±0.40 115.4%***	2.9±0.17 93.3%***	1.6±0.18 45.5%*	2.8±0.28 133.3%***	4.0±0.38 100.0%***	2.5±0.25 108.3%***	22.1±1.91 101.0%***
VLB (1.8mg) **% change**	5.3±0.32 152.4%***	5.5±0.37 189.5%***	3.2±0.32 113.3%***	2.1±0.30 91.0%**	3.3±0.20 175.0%***	5.1±0.27 155.0%***	3.0±0.28 150.0%***	27.5±2.38 150.0%***

The % changes were calculated from control.

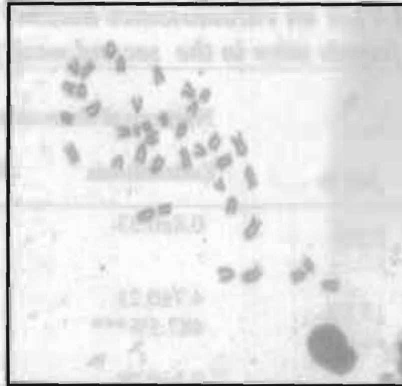


Fig. (4): Cell metaphase of negative control in mouse bone marrow cells.

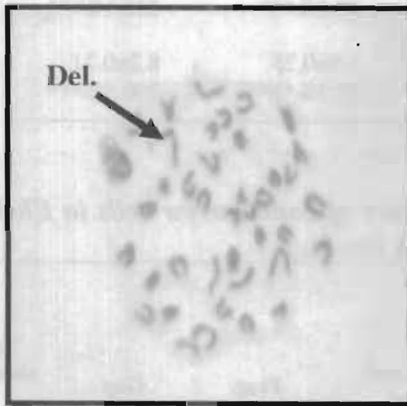


Fig. (5): Deletion metaphase induced by CDDP (3mg) in mouse bone marrow cells.

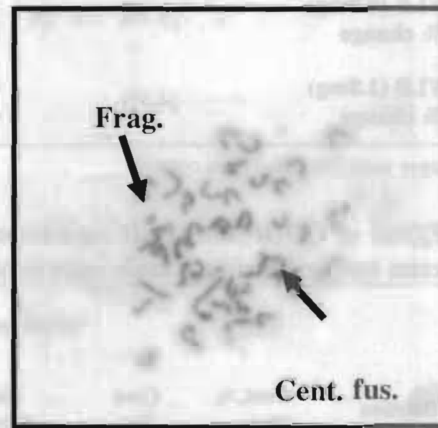


Fig. (6): Fragment and centromeric fusion in metaphase induced by CDDP (6mg) in mouse bone marrow cells.

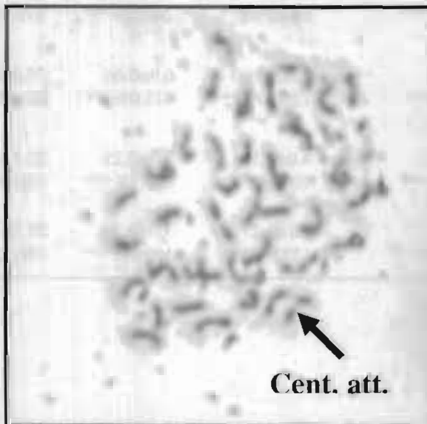


Fig. (7): Centromeric attenuation metaphase induced by VLB (0.9mg) in mouse bone marrow cells.

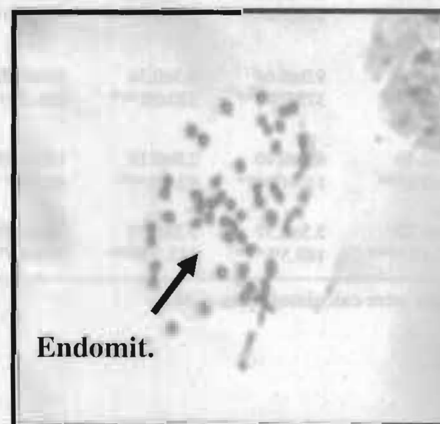


Fig. (8): Endomitosis metaphase induced by VLB (1.8mg) in mouse bone marrow cells.

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الملخص العربي

تأثير بعض العقاقير المضادة للسرطان على DNAوكروموسومات إناث الفئران الحاملة لخلايا Ehrlich ascites tumor

منى هاشم حسين*-زكريا أحمد طلب**-رضا عبد الحليم مغيب*-ماجد إبراهيم إبراهيم**

*قسم الوراثة- كلية الزراعة-جامعة القاهرة-الجيزة-مصر

** شعبة التقييم الدوائى الجزيئى-الهيئة القومية للرقابة والبحوث الدوائية- الجيزة-مصر

تهدف هذه الدراسة إلى معرفة التغيرات الكيميائية والوراثية للأدوية المضادة للسرطان كالسسبلاتين والفنبلاستين على الأنشطة المضادة للأكسدة، DNA وكروموسومات خلايا نخاع العظام في الفئران الإرليش الصغيرة البيضاء. حيث تم استخدام السسبلاتين بتركيز (3.6 مجم/كجم وزن الجسم) وكذلك والفنبلاستين بتركيز (1.8,0.9 مجم/كجم وزن الجسم) وذلك كجرعة واحدة أسبوعيا لمدة أسبوعين. وبعد 24 ساعة من المعاملة بهذه العقاقير وجد إن كلا منهما أدى إلى زيادة تركيز المالون ألدهيد. أيضا أدت هذه العقاقير إلى انخفاض تركيز كل من البروتين, الجلوتاثيون في مستخلص الكبد المتجانس. كذلك أوضح الفصل الكهربي لبروتينات السيرم وجود اختلافات في بعض حزم البروتينات لهذه المعاملات مقارنة بالمجموعة الضابطة. كما أدت هذه العقاقير إلى انخفاض وزن جسم وكبد الفئران وكذلك تركيز DNA في أنسجة الكبد وزيادة الانحرافات الكروموسوميةالعددية و التركيبية لخلايا نخاع العظام في الأسبوع الثاني من المعاملة مقارنة بالمجموعة الضابطة. كما وجد أن تأثير هذه العقاقير يعتمد على الجرعة المعطاة للحيوان.