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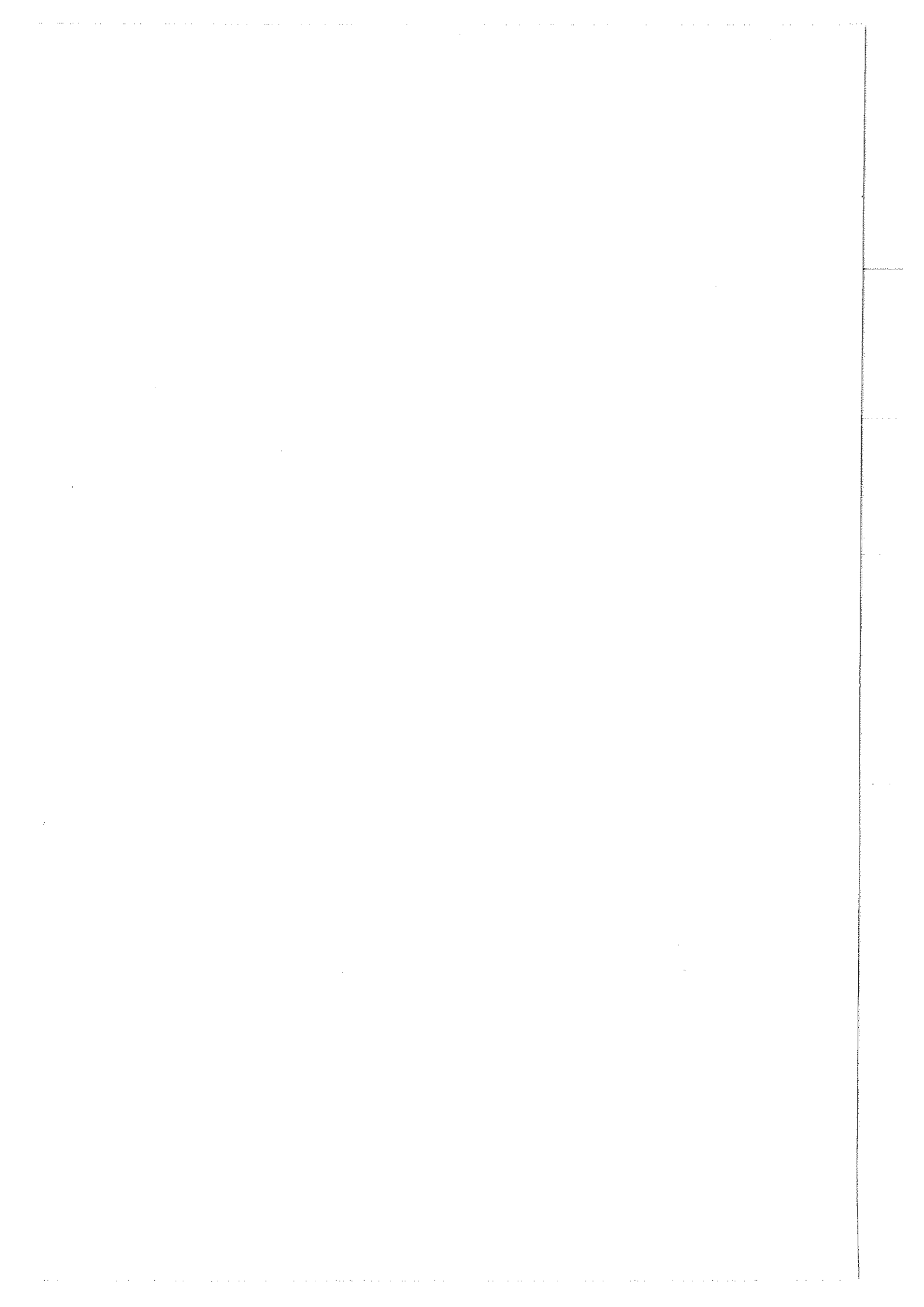
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## Protective Effect Of Ascorbic Acid On Cisplatin Genotoxicity In Male Mice Bone Marrow Cells

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### Abstract

Cisplatin (cis-diamminedichloroplatinum II) is an effective antitumor agent with a wide spectrum of activity against various solid tumors, but it has serious side effects on nontumor cells. Cisplatin produces intra- and interstrand DNA cross-linking effects and chromosomal aberrations in mammalian cells. Vitamin C (ascorbic acid) is an antioxidant that can scavenge free radicals and protect cellular macromolecules, including DNA, from oxidative damage induced by different agents. Pretreatment administration of ascorbic acid on cisplatin induced chromosome aberrations has been determined in bone marrow cells of Swiss albino mice. Results showed that cisplatin (7.5 & 10mg/kg bw) IP injection to male mice induced significant increase in the frequencies of chromosomal aberrations. The results of pre-treatment with ascorbic acid (66mg/kg bw) showed a significant decrease in the number of chromosomal aberrations induced with cisplatin tested doses. Ascorbic acid did not exhibit any clastogenic effect in male mice bone marrow cells. We concluded that ascorbic acid has a protective role against the genotoxicity induced by antitumor drug cisplatin.

**Key words :** Cisplatin- Antioxidants- Ascorbic Acid-Chromosomal Aberration- genotoxicity - Bone Marrow Cells- Mice.

### Introduction

Cisplatin (cis-diamminedichloroplatinum II) is an effective antitumor agent with a wide spectrum of activity against various solid tumors, but it has serious side effects on nontumor cells, including free radical generation (Masuda *et al.*, 1994). Cisplatin produces intra- and interstrand DNA cross-linking effects (Costa *et al.*, 1996). This cytostatic induces chromosomal aberrations in cultured mammalian cells (Nefic, 2001) mouse bone marrow cells (Wiencke *et al.*, 1979) and peripheral blood lymphocytes in patients (Osanto *et al.*, 1991) and sister-chromatid exchanges in human lymphocytes in vitro (Wiencke *et al.*, 1979 ; Bradley *et al.*, 1979) and in vivo (Wiencke *et al.*, 1979). The frequency of occurrence of cells with micronuclei is increased by cisplatin in human lymphocytes in vitro (Gebel *et al.*, 1997 ; Nefic,

2001) and in erythrocytes in bone marrow cells of mice (Nakagawa *et al.*, 1995). Identification and analysis of agents with anticlastogenic activity that reduce the frequency of chromosomal aberrations and possibility of practical application of natural protectors against the clastogenic (and mutagenic/ carcinogenic) action of chemical mutagens have a great importance (Nefic, 2001). Ascorbic acid (AA) is an essential micronutrient for man, with many biological roles. It is a powerful antioxidant both directly via scavenging of reactive oxygen species and indirectly through regeneration of other antioxidant systems (Griffithsa and Lunecb, 2001). Many in vivo assays found that Vitamin C successfully reduced the clastogenic effects of many antitumor agents. Vitamin C inhibited tumor cell growth, binding of the

active carcinogenic metabolite to cellular DNA (Gentile *et al.*,1998). As an antioxidant, Vitamin C may also have a potential anticancer activity, but its possible role and its mechanism of action in cancer prevention has not been clearly established ( Nefic, 2001). Therefore, the present study was undertaken to investigate the protective effects of Vitamin C on cisplatin induced chromosomal aberrations in mice bone marrow cells.

### Materials And Methods

This experiment was carried out on male Swiss albino mice 10-12 weeks old and weighing approximately 30g. Animals were kept and bred in an environmentally controlled room with a temperature of 23± 1. Chow and water were available *ad libitum*. Each experimental group was consisted of six animals for each treatment and control as shown in table (1).

Doses of cisplatin were selected on the basis of its effectiveness in inducing chromosomal aberration (Weijl *et al.*, 1997). The dose of ascorbic acid was calculated according to the therapeutic dose and converted to mice according to Paget and Barner , (1967) . Ascorbic acid was administered by gavage, 24 hrs prior to the injection of IP single dose of cisplatin. Animals of control group were treated IP with saline in the same volume. All animals were injected IP with colchicines 2 hrs before sacrifice to arrest the cell division at metaphase.

#### Scoring Of Slides

Bone marrow preparation for the analysis of chromosomal aberrations in

metaphase cells were obtained by Yosida and Amano, (1965). One hundred metaphases per animal were analyzed in order to determine the frequencies of different chromosomal aberrations.

#### Statistical Analysis

Data of chromosomal aberrations examination were analyzed statistically using one way analysis of variance (ANOVA) according to Gupta (1995).

### Results

The types of structural and numerical chromosomal aberrations induced by cisplatin and their frequencies in male mice bone marrow cells (at the concentration of 7.5 and 10mg/kg body weight) in the absence and the presence of Vitamin C (at the concentrations of 66 mg/kg B.w.) are presented in Table (2) & Fig. (1). Results showed that the treatment with Vitamin C alone at the tested concentration did not change the frequencies of chromosomal aberrations when compared with the untreated control. Intra-peritoneal injection with Cisplatin alone 7.5 mg & 10 mg/kg bw induced a statistically significant increase in the frequencies of total chromosomal aberrations ( $P < 0.001$ ) when compared with control. The results also showed that Vitamin C pre-treatments significantly decreased the number of total chromosomal aberrations induced by Cisplatin ( $P < 0.001$ ) in male mice bone marrow cells when compared with the animals treated with cisplatin alone. However, the number of these aberrations was not reduced to the control number of chromosomal aberrations as shown in Fig. (1).

Table (1): Experimental groups and treatment protocol.

Treatments	Groups	Dose
Control	I	vehicle only(dist. water)
Ascorbic acid	II	66 mg/kg b.w
Cisplatin	III	7.5mg/kg b.w
Ascorbic acid + cisplatin	IV	66 mg/kg + 7.5mg/kg b.w.
Cisplatin 10mg/kg	V	10 mg/kg bw
Ascorbic acid + cisplatin	VI	66 mg/kg + 10 mg/kg bw

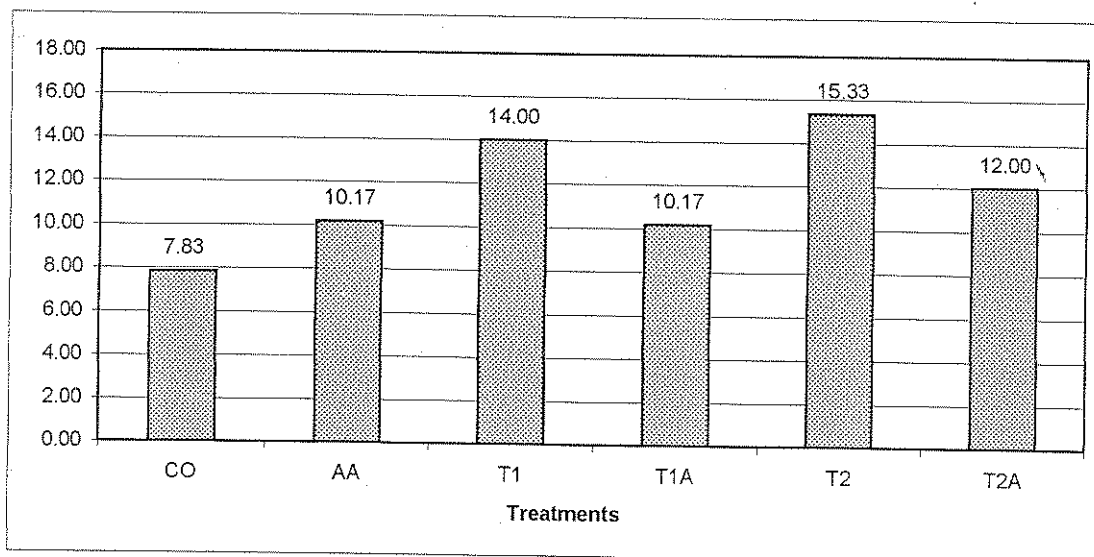
**Table 2 : Frequencies of different chromosomal aberrations in male mice bone marrow cells of all experimental groups.**

Groups	Numerical aberrations		Structural aberrations							Total aberrations
	2n-	2n+	deletion	Chromatid gap	Isochromatid gap	Chromatid break	Isochromatid break	Centromeric attenuation	fragment	
Control	0.50	0.00	0.50	2.67	0.50	1.17.05	0.00	0.83	1.67	7.83
	± 0.55	± 0.00	± 0.84	± 1.03	± 0.55	± 0.41	± 0.00	± 0.75	± 0.52	± 2.14
Ascorbic acid	1.33	0.17	1.33	1.50	0.17	2.33	0.83	1.67	0.83	10.17
	± 1.20	± 0.41	± 0.82	± 1.05	± 0.41	± 1.03	± 0.75	± 1.03	± 0.75	± 1.60
7.5mg/kg Cisplatin	1.17	0.33	2.17*	2.83	1.83*	1.67	1.33*	0.83	1.83	14.00**
	± 0.75	± 0.52	± 0.75	± 1.60	± 0.75	± 1.37	± 0.82	± 0.75	± 0.76	± 2.83
Ascorbic acid + 7.5mg/kg Cisplatin	1.00	0.17	0.17	2.33	0.17	2.33	0.17	2.33*	1.50	10.17
	± 0.63	± 0.41	± 0.41	± 1.03	± 0.41	± 1.03	± 0.41	± 1.21	± 1.05	± 2.40
10mg/kg Cisplatin	1.00	0.50	2.30*	2.83	1.00*	2.30	0.33	3.00**	2.00	15.33**
	± 0.63	± 0.55	± 1.37	± 1.94	± 0.89	± 0.52	± 0.52	± 1.79	± 0.89	± ±2.25
Ascorbic acid+ 10mg/kg Cisplatin	1.00	0.17	1.33	2.83	1.50*	2.00	1.33*	0.83	1.00	12.00**
	± 0.63	± 0.41	± 0.52	± 0.98	± 0.55	± 1.41	± 0.82	± 0.98	± 1.26	± 2.37

Data represent the mean ± standard error of each chromosomal aberrations in six animals.

\* statistically significant at  $p \leq 0.05$ .

\*\* statistically significant at  $p \leq 0.01$ .



**Fig. (1): Histogram Showing the reduction in cisplatin induced genotoxic damage in presence of ascorbic acid.**

CO	Control
AA	Ascorbic Acid
T1A	Cisplatin 7.5 mg+ Ascorbic Acid 66 mg
T1	Cisplatin 7.5 mg
T2A	Cisplatin 10 mg+ Ascorbic Acid 66 mg
T2	Cisplatin 7.5 mg

## Discussion

The use of natural dietary antioxidants to prevent antitumor agents -induced chromosomal damage is currently eliciting considerable interest. Antitumor agents are known to interact with specific biological molecules, and evidence has been obtained that treatment with antitumor drugs from different categories leads to generation of free radicals in nontumor cells both in vivo and in vitro ( Weijl *et al.*,1997). Cisplatin is an effective antitumour drug but it has serious side effects on non tumour cells, including free radical generation (Masuda *et al.*, 1994). Cisplatin produces intra- and interstrand DNA cross-linking effects (Costa *et al.*, 1996). This cytostatic drug induces chromosomal aberrations in cultured mammalian cells (Nefic, 2001), mouse bone marrow cells (Wiencke *et al.*, 1979) and peripheral blood lymphocytes in patients (Osanto *et al.*, 1991) and sister-chromatid exchanges in human lymphocytes in vitro and in vivo (Bradley *et al.*, 1979). The frequency of occurrence of cells with micronuclei is increased by cisplatin in human lymphocytes in vitro (Gebel *et al.*, 1997) and in erythrocytes in bone marrow cells of mice (Nakagawa *et al.*, 1995). Results of the present study markedly showed that a single IP injection of both doses (7.5 and 10 mg/kg body weight) of Cisplatin significantly increased the frequencies of chromosomal aberrations in mice bone marrow cells. The chromosomal aberrations pattern revealed that chromatid breaks and gaps occurred more frequently. However, the results of pre-treatment with Vitamin C indicated that the number of

aberrant metaphases induced by cisplatin in mice bone marrow cells were statistically significantly decreased. Vitamin C shows most efficient anticlast-ogenic effect in the treatments with cisplatin. This result was in agreement with (Giri *et al.*, 1998) they suggested that vitamin C has a definite protective role on chromosomal aberrations and micronucle formation induced by cisplatin. Vitamin C also has in vivo anticlastogenic effects against chromosomal damage and number of abnormal metaphases induced by cisplatin in rodents (Gentile *et al.*, 1998; Antunes *et al.*, 2000). Vitamin C successfully reduces the clastogenic effect of many other antitumour drugs like cyclophosphamide (Ghaskadbi *et al.*, 1992), doxorubicin (Antunes and Takahashi, 1998, 1999) and bleomycin (Anderson *et al.*, 1994 & 1995). Also, Vitamin C reduces the chromosomal aberrations induced by trenimon (Gebhart, 1984), H<sub>2</sub>O<sub>2</sub> (Dallapiccola, 1985), ethyl methanesulfonate (Kuroda, 1987; Kojima, *et al.*, 1992), levodopa, noradrenalin and dopamine (Iwawaki, *et al.*, 1988) and pesticides (Khan and Sinha, 1993) and sister chromatid exchanges (SCEs) induced by cyclophosphamide, mitomycin C (Krishna *et al.*, 1986), thiotepa, l-ethionine (Lialiaris *et al.*, 1987). Odin (1997) showed that continual oral consumption of large amounts of Vitamin C for a long period is able to significantly decrease the number of chromosomal breaks induced in human lymphocytes by various xenobiotics. Similar results were obtained by Duthie *et al.* (1996). They showed that supplementation of the diet with Vitamin C results in a highly significant decrease in

endogenous oxidative base damage in the DNA of lymphocytes of patients, and lymphocytes of antioxidant-supplemented subjects showed an increased resistance to oxidative damage in vitro. The protective role of Vitamin C observed here suggests that Vitamin C has an antioxidative effect on damage induced by free radicals generated during the metabolic activity of cisplatin in mice bone marrow cells. Thus, the present study clearly indicates the protective role of vitamin C against cisplatin induced genotoxicity.

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## التأثير الوقائي لحمض الاسكوربيك على السمية الوراثية لعقار السيسبلاتين في

### نخاع عظام ذكور الفئران

ندى النواتى

قسم البيولوجى - جامعة الملك عبدالعزيز

تهدف هذه الدراسة إلى توضيح دور حمض الاسكوربيك فى الوقاية من التأثيرات السمية الوراثية لعقار السيسبلاتين المضاد للسرطان.

استخدم فى هذه الدراسة ٣٦ فأر أبيض عمر ١٠ - ١٢ اسبوع وقسمت الحيوانات الى ٦ مجاميع فى كل مجموعة ٦ فئران.

حقنت المجموعة الأولى بماء مقطر واعتبرت المجموعة الضابطة بينما الثانية حقنت ب ٦٦ ملجم / كجم من حامض الاسكوربيك واعتبرت المجموعة الضابطة الموجبة وحقنت المجموعة الثالثة والخامسة بمركب السيسبلاتين ٧,٥ ، ١٠ ملجم / كجم على التوالي وأخيراً المجموعة الرابعة والسادسة تم حقنهم ٦٦ ملجم / كجم حامض الاسكوربيك بالإضافة الى ٧,٥ ، ١٠ ملجم سيسبلاتين على التوالي.

وقد أوضحت النتائج المتحصل عليها التأثير الوقائي لحامض الاسكوربيك على السمية الوراثية لمركب السيسبلاتين حيث انخفض معدل التشوهات الكروموسومية عند إعطائه مع عقار السيسبلاتين مقارنة بالمجموعة التى أعطيت السيسبلاتين فقط وهذا دائماً يدل على التأثير الوقائي ضد التشوهات الكروموسومية لحمض الاسكوربيك فى خلايا نخاع العظام.

