

Role of vitamin E-acetate on cisplatin induced genotoxicity: An *in vivo* analysis

Research Article

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Abstract: Vitamin E has generated immense interest because of its potential of being an antioxidant, a neuroprotector, and a protector against atherosclerosis, carcinogenesis and cardiovascular disease. However, the prooxidant chemistry of vitamin E cannot be ignored since it is related to the generation of peroxy radicals. In the present study, 125, 250 and 500 mg/kg of vitamin E-acetate (VE) administered intraperitoneally (i.p.) to Balb/C mice significantly induced 6%, 8% and 11.33% (control value=2.33%) of chromosome aberrations (CA) and 0.88%, 1.39% and 1.81% (control value=0.61%) of micronucleus (MN), following 24 hour of treatment in the bone marrow cells. In the germ cells, VE did not induce any sperm head abnormality (SHA) after 35 days of exposure. Most importantly, it has been observed that pre-treatment with VE significantly reduces CA, MN, and SHA induction by chemotherapeutic drug cisplatin (CIS). Our findings suggest that lone treatment with VE induce genotoxicity in somatic cells after 24 and 48 hours of exposure but not in germ cells after 35 days of exposure, whereas pre-treatment with VE reduces CIS induced genotoxicity as well as cytotoxicity. There exists a thin line of difference on the behavioral transition of VE when acting alone and when acting with a drug.

Keywords: Vitamin E-acetate • Cisplatin • Chromosomal aberration assay • Micronucleus assay • Sperm head abnormality assay

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1. Introduction

Vitamin E, the most potent lipid peroxy radical scavenger, has profound significance of being an antioxidant, [1] a neuroprotector [2,3], and a protector against atherosclerosis, carcinogenesis [4,5] and cardiovascular disease [6]. It also has the property of being a strong anti-inflammatory agent [7]. Vitamin E has been reported to enhance radiation-exposed genotoxicity [8], cause harmful effects on some cigarette smokers [9] and has antimutagenic potential against the effects of various harmful chemicals, drugs and metal elements. In one study with vitamin E and chemotherapeutic drug Adriamycin it was observed that the lethality of the drug was reduced by administration of vitamin E-acetate, which reduced the drug-induced lipid peroxidation [10]. In another study, vitamin E-succinate enhanced the cytotoxic effects of Adriamycin on human prostate

cancer cells *in vitro* [11]. It has been observed that vitamin E supplementation prior to cisplatin treatment had shown protective role against cisplatin induced nephrotoxicity and neurotoxicity [12,13]. However, there is insufficient data on the effects of vitamin E on cytotoxicity and genotoxicity *in vivo*. Most of the conducted studies on antioxidative/prooxidant effect of vitamin E have been conducted *in vitro*. The little available data and literature on vitamin E genotoxicity and its behavior with chemotherapeutic agents lead to a highly confusing, contradictory and inconclusive scenario. Hence further research needs to be conducted to determine the role of vitamin E when acting with chemotherapeutic agents in order to protect the gene pool from the genotoxic potential of such drugs. This work is an attempt to evaluate the genotoxic potential of cisplatin and the modulatory effects of various doses of vitamin E-acetate with cisplatin in somatic and germ cells *in vivo*.

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2. Experimental Procedures

2.1 Chemicals

Cisplatin (CIS) bearing CAS number 15663-27-1 was obtained from Cadila Pharmaceuticals, India. Colchicine and vitamin E-acetate bearing CAS number 7695-91-2, chemical formula $C_{31}H_{52}O_3$ and molecular weight of 472.76 were purchased from Sigma chemicals Co. (St. Louis, MO). Giemsa stain was purchased from HiMedia Laboratories Pvt. Ltd, India. All other chemicals used were of analytical grade. All other chemical solutions were freshly prepared in distilled water prior to experimentation.

2.2 Test animals

The experiments were carried out in accordance with the Animal Ethics Committee of Assam University, India. Inbred strain of Balb/C mice (male and female) between 6-8 weeks old, weighing 25-30 gram, maintained at room temperature at $25.0 \pm 5.0^\circ\text{C}$ with 12 h dark and 12 h light cycle were used for the study. The animals were purchased from Pasteur Institute (Shillong, India). They were maintained with standard food pellets and water provided *ad libitum*.

2.3 Dose and treatment

CIS (4 mg/kg b.w.) was used through i.p. route in an aqueous medium. Vitamin E of three different doses (VE1=125, VE2=250, VE3=500mg/kg/b.w) were selected and administered intraperitoneally (i.p) for 5 consecutive days prior to the drug administration. Vitamin

E-acetate was freshly prepared every time in ethanol solvent and the amount of vitamin E was injected based on dose, dilution factor and, most importantly, animal's body weight. The animals tolerated all three doses of anti-oxidants without any physical signs of toxicity.

2.4 Chromosome aberrations (CA) assay

The CA assay was carried out as described by Preston *et al.* [14] and Krishna and Theiss [15] with minor modifications. Experimental animals were injected with 2 mg/kg b.w of colchicine 1.5 h prior to sacrifice following 24 h and 48 h of the treatment of test chemicals. Bone marrow cells were collected by flushing with 0.56% KCl (pre-warmed at 37°C) from femur bone and incubated for 20 min at 37°C . The material was centrifuged at 1000 rpm for 5 min, fixed with aceto-methanol (1:3) followed by refrigeration for 30 min. The material was centrifuged and re-suspended in fresh aceto-methanol. The slides were prepared by dropping the sample on chilled slides and running over the flame. Staining was done in 5% buffered Giemsa stain (pH 7.0) after 24 h, air-dried and covered with cover slips. At least 100 well-spread metaphase plates were studied per animal for CA as indicated in Table 1 and 2. By this technique we can observe under microscope genetic disturbances like breaks, gaps, exchanges, robertsonian translocation, sister chromatid union and many more additional types (Figure 1). The mitotic index was calculated from a scan of 2000 cells per animal. Mitotic index for cytotoxicity evaluation was calculated as percentage of dividing cells out of total bone marrow

Treatment	Route	Total Cells (n)	Aberrent cells (Total)	% Aberrant cells $\pm$ S.D	Chromatid Break/Gaps	Ischromatid Breaks/Gaps	Exchanges	SCU	R.T	Total Aberrations mean $\pm$ SD (excluding gaps)	Total Aberrations mean $\pm$ SD (including gaps)	Suppression (%)
Con	i.p	305/3	8	2.61 ± 0.48	7	1	0	0	0	2.30 ± 0.54	2.62 ± 0.48	
VE1	i.p	300/3	21	7.00 ± 2.36	6	4	2	0	5	6.00 ± 1.78	7.33 ± 1.25	
VE2	i.p	300/3	22	7.33 ± 1.86	19	6	1	2	4	$8.00 \pm .54^{**}$	$10.33 \pm 2.25^{***}$	
VE3	i.p	300/3	28	9.33 ± 0.51	18	1	7	8	4	$11.33 \pm 1.36^{***}$	$15.32 \pm 2.25^{***}$	
CIS	i.p	300/3	73	24.33 ± 3.14	61	4	6	1	7	33.33 ± 2.25^{ab}	35.00 ± 2.68^f	
VE1+CIS	i.p	300/3	75	25.00 ± 1.54	105	4	15	8	2	31.66 ± 0.51^c	33.66 ± 0.51	5.46
VE2+CIS	i.p	300/3	62	20.66 ± 8.45	55	11	14	7	3	$20.66 \pm 8.45^{a*}$	26.66 ± 13.18	17.79
VE3+CIS	i.p	300/3	42	$14.00 \pm 4.09^*$	21	6	4	3	2	$13.00 \pm 3.22^{bc**}$	$16.00 \pm 3.89^{***}$	42.45

Table 1. Frequency of chromosomal aberrations in the bone marrow cells of mice induced by anticancer agent cisplatin (CIS) and protective intervention by various doses of antioxidant vitamin E-acetate (VE) at 24 hour of treatment.

Con - Solvent vehicle was given; CIS - Cisplatin (4 mg/kg/bw); Vitamin E-acetate: VE1=125 mg/kg/bw; VE2=250 mg/kg/bw; VE3=500 mg/kg/bw; i.p - Intraperitoneal; SCU - Sister chromatid union; R.T - Robertsonian translocation. Groups bearing the same superscript are significantly different from each other [b, c, f = $P < 0.01$; a = $P < 0.05$]; Groups bearing the any of the following symbol is significantly different from the Control groups, * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

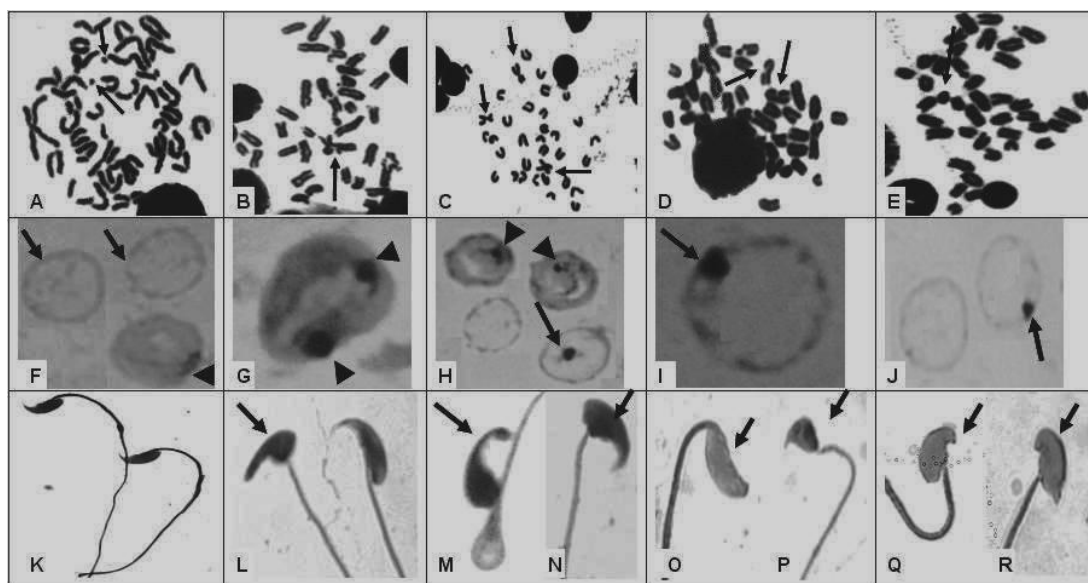


Figure 1. Photomicrographs of murine bone marrow metaphase spreads (A-E), PCE and NCE (F-J) and epididymal sperm cells (K-R). A: Showing metaphase chromosomes with breaks (long arrows). B: Showing chromosomes having exchange (long arrow). C: Showing chromosome with robertsonian translocation (long arrows). D: Showing chromosomes with gaps (long arrows). E: Showing Sister chromatid union (arrow) F: Normal PCE (short arrow) and Normal NCE (long arrows). G: PCE with MN. H: PCE with MN (short arrows) and NCE with MN (long arrow). I-J: NCE with MN (long arrow). K: Sperm with normal head. L: Sperm having beak shaped head (arrow). M: Sperm with hook shaped head. N: Sperm with altered head. O: Sperm having banana shaped head. P: Sperm with a dwarf head shape. Q-R: Sperm with amorphous shaped head.

cells counted. The suppression percentage of CA was calculated as follows:

$100 - (\text{percent incidence of aberrant cells in vitamin E pre-treated and CIS post treated groups} / \text{percent incidence of aberrant cells in only CIS treated group} \times 100)$.

2.5 Micronucleus (MN) assay

After appropriate time of treatment (24 or 48 hour post treatment), the animals were sacrificed by cervical dislocation and MN slides were prepared as described by Schmid [16] with minor modifications. The bone marrow cells were flushed out and collected in 0.9% NaCl (pre-warmed at 37°C). Bone marrow smear was prepared, air-dried and fixed in absolute methanol for 10 minutes. Staining of the slides were done with 5% buffered Giemsa (pH 7.0) on the following day. The slides were mounted in DPX using cover slips. Minimum of 2000 polychromatic erythrocytes (PCE) and corresponding normochromatic erythrocytes (NCE) were scored per animal. The PCEs stain light blue to gray and NCEs stain light pink to light yellow.

2.6 Sperm head abnormality (SHA) assay

The protocol of Wyrobek and Bruce [17] was followed for analyzing sperm head abnormalities. Experimental animals were sacrificed by cervical dislocation after 35 days of treatment. Both the cauda epididymis were dissected out and placed in 1 ml of 0.9% NaCl. The sperms were released by mechanical disruption and

washing of the epididymis. The suspension was sieved through two layers of muslin cloth to remove tissue debris. A drop of the suspension was taken on a clean slide and a smear was made, air-dried, fixed in absolute methanol for 10 minutes. The slides were stained in the following day in 0.1% eosin Y for 10 minutes. One thousand sperm per animal were scored and the abnormalities categorized based on Wyrobek and Bruce categorization of various types of abnormal sperm. The following types of abnormal headed sperm were considered in the present study: Normal, Hookless, Hooked, Giant, Dwarf, Altered head, Triangular, Banana, Needle, Amorphous and Beaked.

2.7 Statistical analysis

ANOVA was used to determine the significance of genetic parameters. Pair wise comparison of significance between the different groups was determined using Tukey's test. ANOVA values were calculated using GraphPad Prism Version 4.03 (Graph pad Inc., San Diego, CA, USA).

3. Results

3.1 Chromosome aberration assay

In Table 1 (24 hour study) and 2 (48 hour study), metaphase chromosomes of bone marrow cells of the treated animals were analyzed for various types of

CAs. The major types of aberrations induced by CIS treated groups were breaks, exchanges, robertsonian translocations, gaps, and sister chromatid unions (Figure 1A-E). The most frequent type of aberrations observed was chromatid breaks. The CAs induced by the drug CIS was found to be significantly high ($P<0.001$) compared to the control group.

In the lone vitamin E-acetate treated groups it was observed that the middle (VE2) and highest dose (VE3) of vitamin E-acetate induce significant level of CAs ($VE2=P<0.01$; $VE3=P<0.001$) compared to control group in the 24 hour study. Similarly in the 48 hour study it was observed that the highest dose of vitamin E-acetate (VE3) induced significant level of CAs ($P<0.001$).

In the vitamin E-acetate supplemented groups with CIS it was observed that there was a gradual decline in the incidence of CAs compared to only CIS treated groups in both 24 and 48 hour study. The lowest dose (125 mg/kg/bw) showed no significant reduction in CAs. In 24 hour study the middle dose (250 mg/kg/bw) showed significant reduction ($P<0.05$). Also, in 48 hour study, it showed reduced incidence of aberrations ($P<0.01$). In both 24 and 48 hour study the highest dose (500 mg/kg/bw) showed highest level of reduction ($P<0.01$), which clearly indicates that there is a dose-dependent decrease in CA index. The percent of aberrant cells also declined with increase in dose and the suppression percentage was found to be 5.46%, 17.79% and 42.45% in 24 hour study and 5.35%, 37.51% and 41.05% in the 48 hour study, respectively (Table 1 and 2).

3.2 Micronucleus assay

MN study was conducted based on the incidence of the frequency of MN in PCES' and cytotoxicity study was based on the ratio of PCE/NCE. It was observed that CIS (4 mg/kg/bw) induced significant level ($P<0.001$) of MN in PCEs in both 24 and 48 hour study (Figure 1G-H). It was also observed that the PCE/NCE ratio was found to be statistically significant ($P<0.05$ in 24 hour study) when compared to control groups (Table 3).

In the 24 hour vitamin E-acetate treated groups, it was observed that the middle (VE2) and highest dose (VE3) of vitamin E-acetate induced significant level of MN in PCEs' ($VE2=P<0.01$; $VE3=P<0.001$) compared to control groups. So, also in the 48 hour study it was observed that the highest dose of vitamin E-acetate (VE3) induced significant MN in PCEs' ($P<0.05$).

In combination treatment with vitamin E-acetate and CIS, it was observed that with the increase in vitamin E-acetate doses there was gradual decline in the incidence of MN. In both 24 and 48 hour study it was observed that the middle and the highest dose of vitamin E-acetate generated protection to CIS induced genotoxicity from 4.28 ± 0.22 (only CIS treated, 24 hour) to 2.81 ± 0.21 and 1.15 ± 0.08 ($P<0.001$) respectively and from 3.30 ± 0.20 (only CIS treated, 48 hour) to 2.58 ± 0.18 ($P<0.01$) and 0.76 ± 0.13 ($P<0.001$) respectively. A 24 hour comparative study between the different vitamin E-acetate treated groups indicate that there is significant difference in decline ($P<0.001$) of MN between low, middle and high dose of vitamin E-acetate. Also, in 48 hour study, it was observed that

Treatment	Route	Total Cells (n)	Aberrant cells (Total)	% Aberrant cells $\pm$ S.D	Chromatid Break/Gaps		Isochromatid Breaks/Gaps		Exchanges	SCU	R.T	Total Aberrations mean $\pm$ SD (excluding gaps)	Total Aberrations mean $\pm$ SD (including gaps)	Suppression (%)
Con	i.p	305/3	8	2.61 $\pm$ 0.48	4	4	0	7	0	0	0	2.30 $\pm$ 0.51	2.30 $\pm$ 1.05	
VE1	i.p	300/3	10	3.33 $\pm$ 1.36	3	3	1	0	3	2	0	3.00 $\pm$ 0.89	4.00 $\pm$ 1.54	
VE2	i.p	300/3	13	4.32 $\pm$ 1.35	12	3	1	1	0	2	0	5.00 $\pm$ 1.54	6.33 $\pm$ 2.87	
VE3	i.p	300/3	28	9.33 $\pm$ 0.51	20	1	9	8	4	3	1	12.66 $\pm$ 0.51***	15.66 $\pm$ 1.86***	
CIS	i.p	300/3	56	18.66 $\pm$ 1.03	32	5	6	0	5	7	9	19.66 $\pm$ 1.86 ^{de}	21.33 $\pm$ 2.87 ^{gh}	
VE1+CIS	i.p	300/3	53	17.66 $\pm$ 0.51	30	2	10	1	4	5	4	17.66 $\pm$ 0.51	18.66 $\pm$ 0.51	5.35
VE2+CIS	i.p	300/3	35	11.65 $\pm$ 2.87**	18	1	7	4	4	3	3	11.66 $\pm$ 2.87 ^{d**}	13.33 $\pm$ 2.25 ^{g**}	37.51
VE3+CIS	i.p	300/3	33	11.00 $\pm$ 0.89**	15	1	6	2	4	4	4	11.00 $\pm$ 0.89 ^{e**}	12.00 $\pm$ 1.78 ^{h**}	41.05

Table 2. Frequency of chromosomal aberrations in the bone marrow cells of mice induced by anticancer agent cisplatin (CIS) and protective intervention by various doses of antioxidant vitamin E-acetate (VE) at 48 hour of treatment.

Con - Solvent vehicle was given; CIS - Cisplatin (4 mg/kg/bw); Vitamin E-acetate: VE1=125 mg/kg/bw; VE2=250 mg/kg/bw; VE3=500 mg/kg/bw; i.p - Intraperitoneal; SCU - Sister chromatid union; R.T - Robertsonian translocation. Groups bearing the same superscript are significantly different from each other [d, e, g, h = $P<0.01$]. Groups bearing the any of the following symbol is significantly different from the Control groups, ** $P<0.01$; *** $P<0.001$.

Treatment	Route	Total PCE studied/n	Total PCE with MN	PCE with MN Mean $\pm$ SD	Total NCE studied	PCE/NCE Mean $\pm$ SD
24 Hour study:						
Con	-	6005/3	37	0.61 $\pm$ 0.20	4135	1.40 $\pm$ 0.02
VE1	i.p	6000/3	53	0.88 $\pm$ 0.11	4045	1.48 $\pm$ 0.06
VE2	i.p	6001/3	84	1.39 $\pm$ 0.23**	4279	1.39 $\pm$ 0.02
VE3	i.p	6000/3	93	1.81 $\pm$ 0.06***	3953	1.52 $\pm$ 0.08
CIS	i.p	6000/3	257	4.28 $\pm$ 0.22***	2705	2.21 $\pm$ 0.18*
VE1+CIS	i.p	6000/3	234	4.06 $\pm$ 0.15 ^{ab}	2852	2.10 $\pm$ 0.11
VE2+CIS	i.p	6000/3	169	2.81 $\pm$ 0.21 ^{ac} ***	3007	1.99 $\pm$ 0.01
VE3+CIS	i.p	6000/3	69	1.15 $\pm$ 0.08 ^{bc} ***	3071	1.95 $\pm$ 0.04
48 Hour study:						
Con	-	6005/3	37	0.64 $\pm$ 0.22	4135	1.46 $\pm$ 0.06
VE1	i.p	6000/3	45	0.75 $\pm$ 0.11	3875	1.54 $\pm$ 0.06
VE2	i.p	6000/3	49	0.81 $\pm$ 0.16	4242	1.40 $\pm$ 0.01
VE3	i.p	6000/3	53	1.05 $\pm$ 0.04*	3998	1.49 $\pm$ 0.08
CIS	i.p	6000/3	198	3.30 $\pm$ 0.20***	2998	2.00 $\pm$ 0.07
VE1+CIS	i.p	6000/3	195	3.24 $\pm$ 0.12 ^{de}	2928	2.05 $\pm$ 0.08
VE2+CIS	i.p	6000/3	155	2.58 $\pm$ 0.18 ^{df} **	3037	1.97 $\pm$ 0.03
VE3+CIS	i.p	6000/3	46	0.76 $\pm$ 0.13 ^{ef} ***	3013	1.99 $\pm$ 0.01

Table 3. Frequency of Micronucleus (MN) in the bone marrow cells of mice induced by cisplatin (CIS) and intervention studies by various doses of antioxidant vitamin E-acetate (VE).

Con - Solvent vehicle was given. CIS - Cisplatin (4 mg/kg/bw); Vitamin E-acetate: VE1=125 mg/kg/bw; VE2=250 mg/kg/bw; VE3=500 mg/kg/bw; i.p - Intraperitoneal; PCE - Polychromatic Erythrocytes; NCE - Normochromatic Erythrocytes; n - Total number of animals; Groups bearing the same superscript are significantly different from each other [a, b, c, e, f = $P < 0.001$; d = $P < 0.01$]; Groups bearing the any of the following symbol are significantly different from the Control groups, * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

the difference between the reduction in MN in the low and middle dose and low and high dose was found to be statistically significant to the level $P < 0.01$ and $P < 0.001$, respectively; the difference between middle and high dose was found to be statistically significant as well ($P < 0.001$). It was also observed that increase in vitamin E-acetate doses gradually improved the ratio of PCE/NCE (Table 3).

3.3 Sperm head abnormality assay

Analysis of abnormal sperm after 35 days of exposure to CIS treated groups for abnormality in head morphology like amorphous, beaked, hooked, hookless, altered head, triangular, banana, giant, dwarf, double-headed, etc. showed significant increase ($P < 0.001$) when compared to control groups. The amorphous form was found to be the most frequent type of abnormality over other types (Figure 1 L-R).

In vitamin E-acetate treated groups as observed in Table 4, there was dominant decline in the SHA with the gradual increase in doses. The lowest dose (125 mg/kg/bw) reduced the percentage of aberrant

sperm from 8.58 $\pm$ 0.16 (only CIS treated) to 6.78 $\pm$ 0.23 ($P < 0.01$) in lowest dose, 5.63 $\pm$ 0.27 ($P < 0.001$) in the middle dose and 4.16 $\pm$ 0.29 ($P < 0.001$) in the highest dose. A comparative analysis of all the doses show that the middle and the highest dose deliver significant reduction in % aberrant sperm compared to the lowest dose to the level $P < 0.01$ and $P < 0.001$ respectively. The highest dose delivers better reduction in abnormal sperm compared to the middle dose ($P < 0.05$).

4. Discussion

In the present work, it was observed that lone treatment with vitamin E-acetate induced significant CAs in the middle (VE2) and high (VE3) dose of vitamin E-acetate (Table 1 and 2). In case of the MN test, the middle (VE2) and high (VE3) doses of vitamin E-acetate induced significant level of MN frequency (Table 3). However, in the SHA test, there was no induction of SHA in all the vitamin E-acetate treated groups (Table 4). This indicates that doses of vitamin E-acetate administered

Treatment	Route	Fixation time (D)	Total sperm cells studied (n)	Total aberrant sperm cells studied	Types of abnormalities studied										% Aberration mean $\pm$ SD
					Amorphous	Triangular	Banana	Beaked	Hooked	Hook less	Altered head	Needle	Dwarf	Giant	
Con	-	35	3005/3	101	50	0	0	27	1	6	16	0	0	1	3.36 $\pm$ 0.64
VE1	i.p	35	3046/3	129	55	0	0	20	20	17	4	0	12	0	4.40 $\pm$ 0.44
VE2	i.p	35	3002/3	94	32	1	2	21	15	9	2	1	8	1	3.13 $\pm$ 0.10
VE3	i.p	35	3012/3	58	14	2	4	6	1	11	12	2	1	1	1.92 $\pm$ 0.54
CIS	i.p	35	3040/3	261	75	0	4	65	18	29	39	0	31	0	8.58 $\pm$ 0.16***
VE1+CIS	i.p	35	3006/3	204	72	1	4	38	15	34	30	1	8	1	6.78 $\pm$ 0.23 ^{ab**}
VE2+CIS	i.p	35	3000/3	169	70	0	1	36	7	23	31	0	2	0	5.63 $\pm$ 0.27 ^{ac***}
VE3+CIS	i.p	35	3000/3	134	50	2	2	28	6	21	15	0	2	0	4.16 $\pm$ 0.29 ^{bc***}

Table 4. Frequency of sperm head abnormality (SHA) in mice induced by cisplatin (CIS) and intervention with various doses of antioxidant vitamin E-acetate (VE).

Con - Solvent vehicle was given. CIS - Cisplatin (4 mg/kg/bw); Vitamin E-acetate: VE1=125 mg/kg/bw; VE2=250 mg/kg/bw; VE3=500 mg/kg/bw; i.p - Intraperitoneal; D - days. Groups bearing the same superscript are significantly different from each other [b = $P < 0.001$; a = $P < 0.01$; c = $P < 0.05$]; Groups bearing the any of the following symbols are significantly different from the Control groups, * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

to animals generate differential response to different cell types. It appears that somatic cells were more vulnerable to high doses of vitamin E-acetate than the germ cells, which were more resistant towards the chemical. It is believed that germ cells are highly specialized cells that have greater longevity and possess high genomic stability compared to somatic cells [18,19]. If that is the case, there appears to be some unknown mechanism by which the germ cells surpass the damage induced by higher doses of vitamin E-acetate. However, further research and studies need to be conducted on the somatic and germ cells and their response towards vitamin E-acetate to derive any conclusions. However, the results indicate that there are various aspects to the biochemistry and behavior of vitamin E-acetate which, in addition to its antioxidative properties, may also have the attribute of genotoxic potential based on the doses and duration employed.

In the present study, it was quite interesting to observe that doses of vitamin E-acetate was found to be genotoxic to somatic cells when administered alone, however showed protection against cisplatin-induced genotoxicity in all the three parameters studied. Cisplatin had induced a very significant level of chromosomal aberrations and micronucleus formation in both 24 and 48 hour studies as well as sperm head abnormalities after 35 days of exposure. We know cisplatin is a known anti-tumor

agent which reacts with DNA by various mechanisms. It interacts with DNA to produce cross-linked DNA adducts that activate checkpoint signaling pathways and thereby induces apoptosis [20]. The major DNA adducts are the intrastrand cross-links that form between two adjacent guanines and between an adjacent adenine and guanine. In addition, small quantities of monofunctional adduct, DNA-protein cross-links and DNA-interstrand cross-links are also produced [21]. Free radical formation may be another very important mechanism in the development of cisplatin-induced genotoxicity [22-25]. It was observed that there was a significant decline in the level of chromosomal damage (Table 1 and 2), MN index (Table 3) and SHA (Table 4), mostly at the higher doses of vitamin E-acetate ($P < 0.001$). The data suggest that there is a dose dependent suppression of genotoxicity induced by cisplatin with vitamin E-acetate supplementation. It is difficult to justify such findings. However, considering the biochemistry and behavior of both this chemicals, it can be assumed that vitamin E-acetate when acting alone releases peroxy radicals capable of inducing genetic damage in absence of sufficient and proper amounts of radical stabilizing counterparts. However, when both of these agents are administered one after another, there is a probability that radical generation could be inhibited as one counterpart is capable of stabilizing the other through redox reactions.

Previous work on vitamin E had shown that there is a dose related effectiveness of the chemical against oxidative stress in a linear trend with maximum reduction at higher doses [26]. Similarly, in our study, it was observed that there was also dose dependent linear response of vitamin E-acetate with the highest dose (VE3), providing maximum protection ($P < 0.001$). We have also observed in all our time-dependent studies (24 hour and 48 hour) that there is a correlation between changes in the level of genotoxicity/genoprotectivity induced by the chemicals over time. The chromosomal aberration and micronucleus pattern revealed that the total number of aberrant metaphases was noticed to be highest at 24 h of treatment, which decreased appreciably during 48 h. It has been reported that chemicals in general produce the highest frequency of aberrations in rodents 24 h after single exposure, which roughly coincides with the normal 22–24 h length of the cell cycle. However, decrease in aberrations during later periods could be due to possibilities such as elimination of damaged cells, the removal of drug from the body, DNA repair process, etc. In fact, an involvement of post-replication repair process in cisplatin-induced DNA damage has been established [27].

Vitamin E had exhibited protection against various toxic elements both *in vivo* and *in vitro*. Its function as a protectant against genotoxicity is dependent upon its ability to break radical-propagated chain reactions. As a result, the formation of the tocopheroxyl radical, the odd-electron derivative of vitamin E, is an inherent part of any vitamin E-based antioxidative reaction [28]. As a matter of fact, lipid-soluble antioxidant, alpha-tocopherol in biological membranes, reacts with many oxidant molecules. It helps in protection of cell membranes from lipid peroxidation by trapping peroxy radicals. It involves the abstraction of a hydrogen atom from the OH group of the tocopherol by a peroxy (oxidant) molecule. Upon the formation of the tocopheroxyl radical from a reaction between vitamin E and an oxidant molecule, the radical formed is free to interact with another peroxy radical. This reaction generates a stable tocopheroxyl radical which does not further propagate radical chains. This tocopheroxyl radical can be regenerated to alpha-tocopherol by an electron donor, like vitamin C, and is thereby able to maintain cellular antioxidant protection over a period of time [5]. This mechanism can be conceptualized to be involved in neutralizing the free radicals generated by our test drug cisplatin and hence involvement of vitamin E-acetate in minimizing DNA damage.

In one previous work with vitamin E (20 mg/kg) at 24 hour pretreatment in mice, the vitamin E was found to deliver 100% protection to the lethal dose of

iron; additionally, when given within 5 to 60 minutes of acute iron intoxication, vitamin E reduced mortality to nearly 75% [29]. Oxidative stress may be involved in chronic renal failure. To illustrate this and to study the role of vitamin E supplementation in this aspect [30], it was established that vitamin E supplementation visibly protected DNA strand breaks in the lymphocytes of dialysis patients after 14 weeks of therapy, indicating that vitamin E is capable of neutralizing the oxidants involved in DNA strand breaks.

In yet another work, application of vitamin E ameliorated the chromium and/or nickel induced oxidative stress in the mouse liver. It prevented lipid peroxidation as well as protected the antioxidative system [31]. It was demonstrated that vitamin E was capable of delivering protection from lipid peroxidation, ionizing radiation and oxidative DNA damage on the human HCC cell line [32]. Vitamin E supplementation in patients treated with cisplatin chemotherapy was found to deliver neuroprotection to cisplatin-induced peripheral neurotoxicity without affecting the drug efficacy [13]. Vitamin E (400 mg/kg/bw), along with vitamin C and vitamin A, was found to inhibit dimethylaminoazobenzene (DAB) induced hepatoma in male albino rats, clearly suggesting that vitamins are capable of preventing oxidative damage associated with cellular damage [33].

In the present work, vitamin E-acetate supplementation studies with cisplatin clearly show that vitamin E-acetate resulted in a very high level of protection to sperm heads against drug-induced toxicity and the highest doses reduced the frequency of SHA to almost 50% when compared to only the drug treated groups (Table 4). This generates the idea that vitamin E-acetate is very potent against the genotoxicity induced by the drug through some probable mechanisms as stated above. Reactive oxygen species, highly active oxidants generated by such drugs, may affect the developing spermatozoa as well. It was stated in one work that intake of lipid soluble antioxidants like alpha-tocopherol protects against the oxidative DNA damage caused by high PUFA diet [34]. In humans it was clearly and strongly demonstrated that supplementation with vitamin E and vitamin C in infertile males can improve semen quality and fertility in patients [35].

Since vitamin E-acetate is an active redox compound its antioxidant or prooxidant properties depends on the available reaction partner, concentration of the reaction partners, and the cellular environment in which the reaction occurs [36,37]. Thus, vitamin E-acetate is expected to require a co-agent to become either beneficial or harmful. There exists a thin line of difference on the behavioral transition of vitamin

E when acting alone and when acting with a drug. There is a possibility that the prooxidant chemistry of vitamin E can be directed to be used as a therapeutic agent against certain somatically derived tumors cells. Needless to say, further research needs to be conducted before any conclusion is stated on this aspect.

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