Dietary Supplementation with Leaf Extract of *Beta vulgaris* L. var. *benghalensis* Hort. in Modifying Cytotoxicity of Lead Subacetate in Mouse *In Vivo*

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A crude extract of leaves of Indian spinach (*Beta vulgaris* L. var. *benghalensis* Hort.) was observed to modify significantly the cytotoxic effects of a known carcinogen, lead subacetate in mice *in vivo*. Laboratory bred male Swiss albino mice were fed by gavaging the crude extract for 7 days daily (1.5 g fresh weight of leaf per kg b.w. of animal). On day 7, mice were injected intraperitoneally with three concentrations of the carcinogen (20, 30, 50 mg/kg b.w.). Chromosomes were studied from bone marrow cells, 24 h after exposure, following colchicine-fixative-air drying-Giemsa schedule. The endopints screened were chromosomal aberrations (CA) and damaged cells (DC). Lead subacetate, given alone, induced both CA and DC in frequencies directly related to the concentration. The leaf extract given alone, did not induce any aberrations. Prior priming with the extract as a dietary supplement reduced significantly the cytotoxic effects of the two lower concentrations of the carcinogen.
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INTRODUCTION

A large number of plant products used in the diet have been classified as chemopreventors of cancer (Block *et al.*, 1992). Of these, green vegetables form a major proportion. Since mutagenesis has been often associated with carcinogenesis, the antimutagenic properties of a large number of leafy vegetables in the diet have been studied (see Ferguson, 1994) after the initial observation by Kada *et al.* (1978). Protection against damage to chromosomes (anticlastogenic effects) was later recorded in mammalian systems following dietary administration of the plant ingredients (Ito *et al.*, 1986; see Sarkar *et al.*, 1996; Sharma, 1995). In the course of screening for chemopreventors of plant origin in the diet, we had observed that crude extract of the leaves of *Beta vulgaris* L. var. *benghalensis* Hort. (Indian spinach beet) modified the effects of chromium salts (Sarkar *et al.*, 1993; 1995).

The present investigation was undertaken to find out if the crude extract could modify the cytotoxic effects of different concentrations of lead subacetate — a known carcinogen, when given as a regular dietary supplement to mice *in vivo*.

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MATERIALS AND METHODS

Preparation of test chemicals. In screening for clastogenic effects, the guidelines for *in vivo* cytogenetic assays in mice were followed (Preston *et al.*, 1987). The toxicant lead subacetate (Pb $(C_2H_3O_2)_2$, $C_4H_{10}O_8Pb_3$, MW 807.75, Merck, India) was dissolved in deionized water and administered intraperitoneally in three concentrations, namely 20, 30 and 50 mg/kg b.w. of mice.

For preparation of crude extract, fresh leaves of spinach-beet (*Beta vulgaris* L. var. *benghalensis* Hort.) were purchased in bulk from the market. The average chemical composition of the leaves (per 100 g edible portion) was: vitamin C, 70 mg; calcium, 380 mg; nicotinic acid, 3.3 mg; riboflavin, 0.56 mg; vitamin A, 9770 I.U.; fibre, 0.7 g; thiamine, 0.26 mg; protein, 3.4 g (according to Aykroyd, 1956). The fresh leaves were macerated in distilled water and the crude extract was used in a final concentration of 1.5 g of leaves per 1 kg b.w. of animal.

Male Swiss albino mice (*Mus musculus*, L. 2n = 40) from the same litter were used. They were bred in the departmental animal house and were 8–10 weeks old with an average weight of 30 g just before the experiment. They were maintained on standard balanced diet (Hindustan Lever Ltd., India) and unlimited water. The cages were kept at a standard temperature of 25° ± 2°C, relative humidity of 60% ± 5% and light cycle of 12 h (light/dark).

Experimental protocol. Four sets of experiments were started (Table 1). Sets I and II were administered distilled water and

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Table 1. Experimental protocol

Set	Treatment	Concentration (mg/kg b.w.)	Duration
I	Negative control (distilled water)	–	24 h
II	Positive control (mitomycin C)	1.5	24 h
IIIA	Spinach beet leaf extract alone	–	7 days
IIIB	Priming with spinach extract for 7 days followed by lead subacetate on day 7	20	24 h
IIIC	Priming with spinach extract for 7 days followed by lead subacetate on day 7	30	24 h
IIID	Priming with spinach extract for 7 days followed by lead subacetate on day 7	50	24 h
IVA	Lead subacetate alone	20	24 h
IVB	Lead subacetate alone	30	24 h
IVC	Lead subacetate alone	50	24 h

mitomycin C (1.5 mg/kg b.w.) respectively as negative and positive control. Set III had four groups. All mice in this set were fed by gavaging aqueous extract of spinach-beet leaves daily for 7 consecutive days. Three groups (III, B, C and D) were then administered intraperitoneally three concentrations of lead subacetate (20, 30, 50 mg/kg b.w.) on day 7, 1 h after priming. Mice in group IIIA were kept as control with the crude extract alone.

In experimental set IV, three groups of mice were injected intraperitoneally lead subacetate in three different concentrations — 20 (IVA), 30 (IVB) and 50 mg/kg b.w. (IVC) respectively.

Five mice were used for each individual experiment. Animals were killed 24 h after the final exposure. Bone marrow chromosomes were prepared following the usual colchicine (0.04%)-hypotonic (0.075 M KCl) — fixative (1:3 acetic-methanol) — flame drying schedule (Preston *et al.*, 1987; Sharma and Sharma, 1994). Slides were then stained in diluted Giemsa stain (1:20), coded and scored blind.

500 well spread metaphase plates were scanned from each set of five mice. The types of chromosomal aberrations were recorded separately as given by WHO (1985) and included chromatid and isochromatid gaps and breaks and rearrangements, e.g. centric fusion, fission and dicentrics. Chromosomal aberrations/cell (CA/cell) were calculated, regarding each chromatid break as one and each isochromatid break or rearrangement as two breaks. Gaps were excluded. For computing the percentage of aberrant metaphases (%DC), all cells with at least one aberration (excluding gaps) were included.

The data were analysed following a modified *t*-test (Fisher and Yates, 1963) and one-way ANOVA (Sokal and Rohlf, 1987) followed by Duncan's multiple range test (Kotz and Johnson, 1982).

RESULTS AND DISCUSSION

In Table 2, sets I, II and IIIA give the results of exposure to the negative control (distilled water), positive control (mitomycin C) and spinach beef leaf extract alone. Compared with the negative control, mitomycin C was highly clastogenic, the frequency of chromosomal aberrations and percentage of damaged cells induced being significantly higher than the negative control. The effects of the crude leaf extract, however, were almost the same as that of the negative control.

Lead subacetate alone induced clastogenic changes (sets IVA, B and C) when observed at 24 h following a single/ exposure. The degree of clastogenicity was directly proportional to the concentration of the chemical and ranged from significant with the lowest concentration (20 mg/kg b.w.) to highly significant with the highest (50 mg/kg b.w.) compared with distilled water.

The clastogenic effects of spinach beet leaf extract, when given as a dietary supplement daily for 7 days, were not significantly higher than the control, indicating that the extract was non-clastogenic (set IIIA).

When the mice were fed by gavaging the crude leaf extract daily for 7 days and then exposed to lead subacetate and observed after 24 h (set III, B, C and D), the frequencies of CA/cell and DC were reduced significantly almost to the level of the extract in the mice exposed to the two lower concentrations of the salt. The reduction was, however, not significant in mice exposed to the highest concentration.

Tables 3 and 4 show one-way ANOVA and Duncan's multiple range tests respectively. With respect to both

Table 2. Effects of lead subacetate alone and in combination with crude extract of spinach beet leaves

Set	Total chromosomal aberrations G′	G″	B′	B″	RR	CA/cell[a]	%DC[b]
I	11	–	5	–	2	0.018 ± 0.019	1.4 ± 1.349
II	30	4	95	2	6	0.222 ± 0.025[c]	18.2 ± 1.135[c]
IIIA	20	–	7	–	2	0.020 ± 0.011	1.8 ± 0.632
IIIB	12	–	9	–	1	0.022 ± 0.011	2.2 ± 0.942
IIIC	14	–	8	–	3	0.028 ± 0.019	2.2 ± 1.135
IIID	30	–	51	–	2	0.110 ± 0.019[c]	9.8 ± 1.988[c]
IVA	17	–	26	–	2	0.060 ± 0.023[c]	4.6 ± 1.890[c]
IVB	20	–	37	–	4	0.086 ± 0.018[c]	5.4 ± 1.632[c]
IVC	22	–	57	–	4	0.130 ± 0.021[c]	11.6 ± 1.837[c]

G′, G″, chromatid and isochromatid gap respectively
B′, B″, chromatid and isochromatid/chromosome break respectively
RR, rearrangement
[a] Mean chromosomal aberration/cell (excluding gap) ± standard deviation
[b] Mean percentage of damaged cells ± standard deviation
[c] $p < 0.001$
Total of 500 cells per treatment per set.

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Table 3. One-way ANOVA to observe significant differences, if any, amongst different treatment sets

Conc. of lead subacetate (mg/kg b.w.)		Source of variance	Degrees of freedom	Sum of squares	mean sum of squares	*F*
20	Breaks/cell	Between groups (different treatment sets)	3	0.013	0.004	20
		Within groups (error)	36	0.009	0.0002	
	% DC	Between groups	3	62	20.667	14.302[a]
		Within groups	36	52	1.445	
30	Breaks/cell	Between groups	3	0.031	0.0103	64.375[a]
		Within groups	36	0.006	0.0001	
	% DC	Between groups	3	100.4	33.467	9.714[a]
		Within groups	36	124.000	3.445	
50	Breaks/cell	Between groups	3	0.104	0.0347	347[a]
		Within groups	36	0.004	0.0001	
	% DC	Between groups	3	845.100	281.700	123.661[a]
		Within groups	36	82.000	2.278	

[a] Significant at both 0.01 and 0.05 level
% DC, percentage of damaged cell.

endpoints screened, namely, CA/cell and % DC, a linear correlation is observed between experimental sets with non-significant clastogenic effects (distilled water alone; spinach beet extract alone and spinach beet extract plus the lower concentrations of lead subacetate). Exposure to the highest concentration of the salt alone gave highly significant changes, which were only partially reduced by priming with the crude extract.

In a series of publications from our group, regular dietary supplementation with crude extracts of plants, including *Phyllanthus emblica* L. fruit and bulb of *Allium sativum*, was recorded to reduce the effects of clastogens significantly in mice *in vivo* (Dhir *et al.*, 1990; Roy *et al.*, 1992; Das *et al.*, 1993). Aqueous crude extract of spinach beet has been observed to reduce clastogenic effects of cyclophosphamide (Abraham *et al.*, 1986) and chromium (Sarkar *et al.*, 1993). Chlorophyll extracted from the leaf was however itself clastogenic (Sarkar *et al.*, 1995).

The present investigation was undertaken to study the chemopreventive effects of these leaves against lead subacetate, which has been found to be carcinogenic in mice following both oral and parenteral administration (IARC, 1980; USEPA, 1986). The observations made here show that this compound is a potent clastogen and can induce significantly high levels of chromosomal aberrations and damaged cells following a single exposure. The protection afforded by crude spinach beet leaf extract when given daily in diet against the two lower concentrations of the compound indicates that such protection does not extend against high concentrations of the toxicant. Such chemoprevention may be attributed to the complex mixture of the leaf extract (Sarkar *et al.*, 1997). The components of the

Table 4. Duncan's multiple range test

Concentrations of lead sub-acetate (mg/kg b.w.)		Ordering of sample means of different experimental sets			
		Negative control	Spinach-beet extract	Extract plus lead subacetate	Lead subacetate
20	CA/cell	0.018	0.020	0.022	0.062
	% DC	1.400	1.800	2.200	4.600
30	CA/cell	0.018	0.020	0.028	0.086
	% DC	1.400	1.800	2.200	5.400
50	CA/cell	0.018	0.020	0.110	0.130
	% DC	1.400	1.800	9.800	11.600

The straight lines denote insignificant differences between the means at $p=0.05$ level.
CA/cell, chromosomal aberrations/cell
% DC, percentage of damaged cells.

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extract include a variety of antioxidants, like vitamins, ascorbic acid, polyphenols and fibres. The interactions between the components of the extract and the toxicant, result in the protective action of the extract against the clastogenic effects of the toxicant, up to a particular level.

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