

Research article

Comparative efficacy of two microdoses of a potentized homeopathic drug, Cadmium Sulphoricum, in reducing genotoxic effects produced by cadmium chloride in mice: a time course study

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Abstract

Background: Cadmium poisoning in the environment has assumed an alarming problem in recent years. Effective antimutagenic agents which can reverse or combat cadmium induced genotoxicity in mice have not yet been reported. Therefore, in the present study, following the homeopathic principle of "like cures like", we tested the efficacy of two potencies of a homeopathic drug, Cadmium Sulphoricum (Cad Sulph), in reducing the genotoxic effects of Cadmium chloride in mice.

Another objective was to determine the relative efficacy of three administrative modes, i.e. pre-, post- and combined pre and post-feeding of the homeopathic drugs. For this, healthy mice, *Mus musculus*, were intraperitoneally injected with 0.008% solution of CdCl₂ @ 1 ml/100 gm of body wt (i.e. 0.8 mcg/gm of bw), and assessed for the genotoxic effects through such studies as chromosome aberrations (CA), micronucleated erythrocytes (MNE), mitotic index (MI) and sperm head anomaly (SHA), keeping suitable succussed alcohol fed (positive) and CdCl₂ untreated normal (negative) controls. The CdCl₂ treated mice were divided into 3 subgroups, which were orally administered with the drug prior to, after and both prior to and after injection of CdCl₂ at specific fixation intervals and their genotoxic effects were analyzed.

Results: While the CA, MNE and SHA were reduced in the drug fed series as compared to their respective controls, the MI showed an apparent increase. The combined pre- and post-feeding of Cad Sulph showed maximum reduction of the genotoxic effects.

Conclusions: Both Cad Sulph-30 and 200 were able to combat cadmium induced genotoxic effects in mice and that combined pre- and post-feeding mode of administration was found to be most effective in reducing the genotoxic effect of CdCl₂ followed by the post-feeding mode.

Background

Cadmium has been known as a toxic agent in environment which has attracted critical attention for its toxic biological effects [1–10]. The adverse effects of cadmium

in biological systems may result in nephrotoxicity, tumorigenicity or carcinogenicity in fowl, mice, rat and human beings [11–22]. Mutagenic effects of cadmium have been extensively studied in microorganisms [23–25] and

in mammals *in vivo* [22,26,27]. However, although cadmium poisoning has become an environmental hazard in recent years, attempts to reduce cadmium induced genotoxicity by any possible antimutagenic agent(s) are very few [28,29].

In the present study two microdoses of a potentized homeopathic drug Cadmium Sulphuricum (Cad Sulph) – 30 and 200 were fed to mice either before or after the injection of 0.008% CdCl₂ @ 1 ml/100 gm of body weight of mouse, to examine if either or both of these potencies could positively modulate the cytogenetic effects produced by CdCl₂. In fact, the impetus of the study was drawn from our findings that potentized homeopathic drug Arsenicum Album (Ars Alb), derived from succussion and dilution of Arsenic trioxide could successfully and favourably modulate cytotoxic and genotoxic effects of Arsenic trioxide [30–35]. A little deviation has been made in the present experimentation; the homeopathic drug used has actually been derived from a different salt of cadmium through dilution and succussion as per the homeopathic procedure and it was examined if this drug had any ameliorating effect on the genotoxicity produced by the injection of CdCl₂, another cadmium salt. Unfortunately, as homeopathic preparations of Cad Chlor-30/200 were not available, it was not possible to compare the effects of Cad Chlor and Cad Sulph in combating cadmium poisoning. Another objective of the study was to test the comparative efficacies of the three modes of treatment, i.e., pre-, post- and combined pre- and post-feeding of the homeopathic drug in combating sub-acute cadmium poisoning.

Materials and Methods

Materials

Inbred lines of mice (*Mus musculus*) cultured and maintained on artificial standard diet and water *ad libitum* (under the supervision of the animal welfare committee, University of Kalyani) were used as materials for the present study. Generally 3–4 months old healthy adult mice of both sexes weighing between 22–28 gms were selected for the purpose.

Methods

Treatment of test chemical

Mice were intraperitoneally injected with a single dose of aqueous solution of CdCl₂ (0.008%) at the rate of 1 ml/100 gms of body weight (i.e. 0.8 mcg/g of bw approx) and were sacrificed at 6 different fixation intervals, viz. at 6 hr, 12 hr, 24 hr, 48 hr, 72 hr and 96 hr.

Administration of potentized homeopathic drug

0.06 ml of each of the 30th and 200th potency of the homeopathic drug Cadmium sulphuricum in liquid (90% ethyl alcohol) prepared by the standard homeopathic

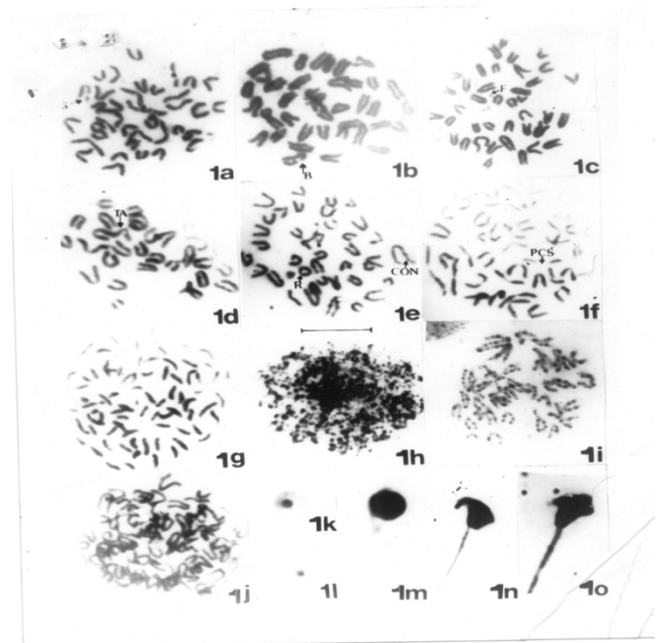
procedure of centesimal dilutions and succussions [32,33] by Hapco, 165 Bipin Behari Ganguly Street, Kolkata, was diluted separately with 20 ml of double distilled water to make stock solutions of the homeopathic drug that was orally administered to experimental mice. In India, these two potencies are most commonly used by the homeopathic practitioners and are easily available in the market.

Experimental design and control

Drug treated series

Drug pre-treated series (T₁–T₂)

1 drop of stock solution (0.06 ml) of the homeopathic drug (both 30th and 200th potencies in separate series) was orally administered to mice with the aid of a pipette every 4 hr prior to the injection of CdCl₂. The respective times of beginning of drug feeding before injection were 6 hr, 12 hr, 24 hr, 48 hr, 72 hr and 96 hr to the sets of mice destined to be sacrificed at 6 hr, 12 hr, 24 hr, 48 hr, 72 hr and 96 hr, respectively. As for example, the mice destined to be sacrificed at 24 hr would start receiving



Photomicrograph I

Photomicrographs different types of chromosome aberrations showing gap (1a), break (1b), fragment of unknown origin (1c), terminal association (1d), ring and constriction (1e), precocious centromeric separation (1f), C-mitotic effect (1g), pulverisation (1h), erosion (1i) and polyplody (1j). 1k-1l: Showing micronucleus in both polychromatic (1k) and normochromatic (1l) erythrocytes. 1m-1o: Showing sperm with abnormal head-shapes (1m to 1o).

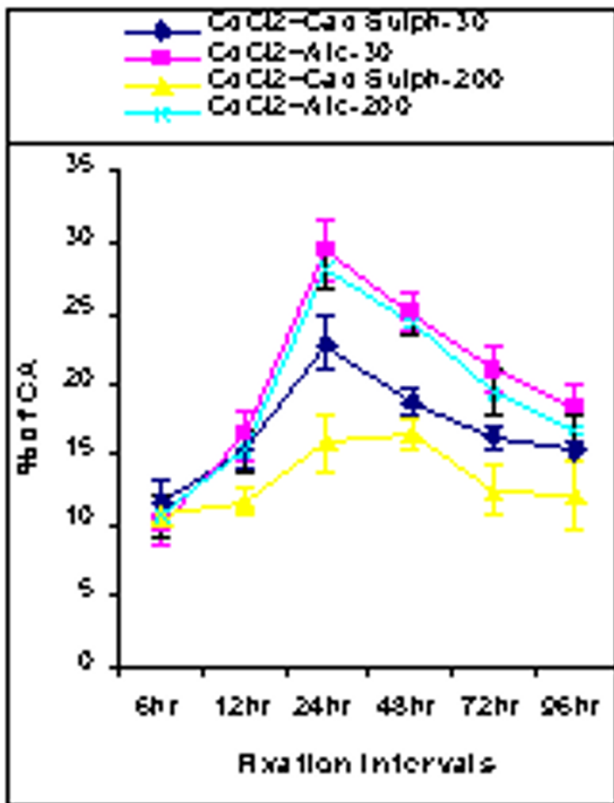


Figure 2 Showing time-dependent frequencies of Cad Sulph-30 pre-fed and Cad Sulph-200 pre-fed series versus their respective CdCl₂ treated alcohol-30 and alcohol-200 control mice. Chromosome aberration (CA)-1; Mitotic index (MI)-2; Micronucleated erythrocytes (MNE)-3 and Sperm head anomaly (SHA)-4.

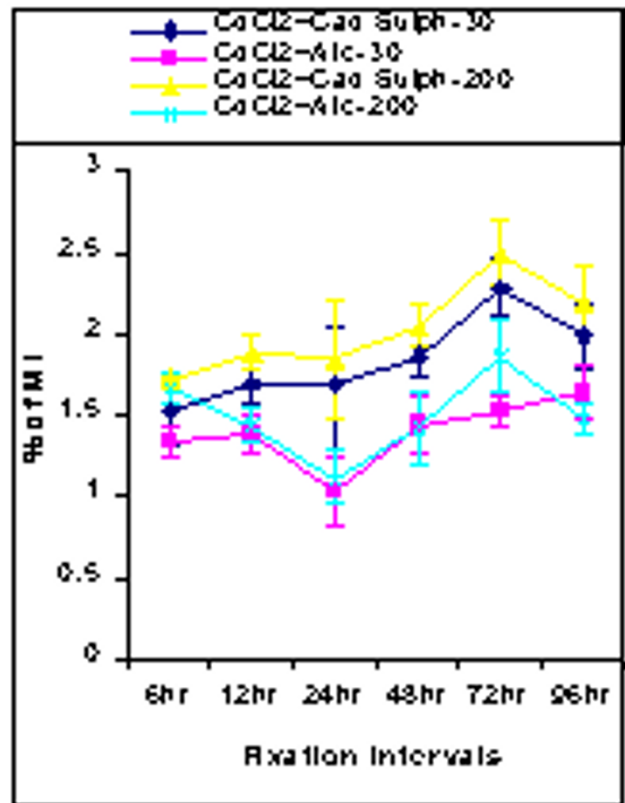


Figure 3 Showing time-dependent frequencies of Cad Sulph-30 pre-fed and Cad Sulph-200 pre-fed series versus their respective CdCl₂ treated alcohol-30 and alcohol-200 control mice. Chromosome aberration (CA)-1; Mitotic index (MI)-2; Micronucleated erythrocytes (MNE)-3 and Sperm head anomaly (SHA)-4.

the homeopathic drug 24 hr before Cadmium chloride injection and those destined to be sacrificed at 48 hr would start receiving the drug 48 hr before injection. In case of pre-fed series, the mice were not fed homeopathic drug after injection. But in the pre- and post-fed combined series, the mice were fed the homeopathic drug both prior to and after the injection. Similarly, in the post-fed series, mice were fed the homeopathic drug only after injection of Cadmium chloride.

Drug post-treated series (T₃-T₄)

Different sets (consisting of 5 mice each) of adult healthy mice were fed 1 drop (0.06 ml) of the stock solution of drug (either 30th or 200th potency) at an interval of 4 hour (during day time only i.e. generally 4 times a day) i.e. the drug was fed twice at an interval of 4 hour to mice sacrificed at 6 hr, thrice to mice sacrificed at 12 hr and 6 times to mice sacrificed at 24 hr and so on, starting at 5 minutes after injection.

Combined pre- and post-drug treated series (T₅-T₆)

In this series different sets of mice (each comprising 5 mice) were fed potentized drug both before and after injection in similar manner as stated above and sacrificed at the 6 different fixation intervals.

Control series (C₁-C₆)

Since the "vehicle" of the homeopathic drug was 90% ethyl alcohol, the same was also potentized to form alcohol-30 and alcohol-200 by the homeopathic procedure of dilutions and succussions. 1 drop (0.06 ml) each of alcohol-30 and alcohol-200 was then diluted separately with 20 ml of double distilled water to produce the stock solutions of succussed alcohol-30 and alcohol-200, respectively. 1 drop (0.06 ml) each of the stock solution of alcohol-30 and alcohol-200 was fed to separate sets of mice (also comprising 5 mice in each set) serving as control at all corresponding intervals of the drug treated series. In earlier experiments in this laboratory another

Table 1: Frequency distribution of Chromosome Aberrations (CA) in 500 bone marrow cells examined (100 cells from each individuals).

Fixation Intervals (hr)	Drug potency	Series	Mitotic Index		Chromosome Aberration					Micronucleated Erythrocyte				Sperm Head Anomaly	
			% ± SE	% of Increased MI	% of Major CA	% of Other CA	% of Total CA ± SE	% of Prot.	% of MN in NCE	% of MN in PCE	P/N	% of Total MN in NCE & PCE	% of Prot.	% of SHA ± SE	% of prot.
6	30	T ₁	1.52 ± 0.20		5.4	6.2	11.6 ± 1.60		0.25	0.99	0.16	0.35 ± 0.04		1.72 ± 0.13	
		C ₁	1.34 ± 0.17	0.18	5.6	4.4	10 ± 1.45	-1.6	0.24	0.78	0.28	0.41 ± 0.04	0.06	1.62 ± 0.12	-0.1
	200	T ₂	1.72 ± 0.02		4.6	6	10.6 ± 1.44		0.18	1.26	0.16	0.33 ± 0.06		1.74 ± 0.09	
		C ₂	1.66 ± 0.16	0.06	4.8	5.8	10.6 ± 1.44	0	0.26	0.76	0.30	0.37 ± 0.04	0.04	1.56 ± 0.23	-0.18
12	30	T ₁	1.68 ± 0.11		6.8	8.4	15.2 ± 1.08		0.23	0.71	0.03	0.33 ± 0.05		1.86 ± 0.13	
		C ₁	1.38 ± 0.12	0.3	8	8.4	16.4 ± 1.75	1.2	0.4	0.6	0.47	0.47 ± 0.04	0.14 ^a	2.04 ± 0.20	0.18
	200	T ₂	1.88 ± 0.11		4.4	7.2	11.6 ± 1.03		0.25	0.77	0.30	0.37 ± 0.04		1.80 ± 0.15	
		C ₂	1.44 ± 0.19	0.44	6.8	8.4	15.2 ± 1.55	3.6	0.51	0.7	0.44	0.57 ± 0.06	0.20 ^a	1.96 ± 0.25	0.16
24	30	T ₁	1.68 ± 0.38		9.4	13.4	22.8 ± 1.98		0.46	1.22	0.38	0.67 ± 0.06		4.40 ± 0.30	
		C ₁	1.04 ± 0.21	0.64 ^a	15.6	13.9	29.4 ± 2.28	6.6	0.84	1.68	0.28	1.03 ± 0.07	0.36 ^b	4.94 ± 0.29	0.54
	200	T ₂	1.84 ± 0.37		8.2	7.6	15.8 ± 1.94		0.59	1.33	0.33	0.73 ± 0.05		4.24 ± 0.30	
		C ₂	1.11 ± 0.17	0.74	14.8	13.4	28.2 ± 1.47	12.4 ^c	0.8	1.51	0.30	0.97 ± 0.10	0.24	4.74 ± 0.21	0.5
48	30	T ₁	1.86 ± 0.13		8.6	10.2	18,80,96		0.84	1.34	0.48	1.01 ± 0.08		5.24 ± 0.38	
		C ₁	1.44 ± 0.17	0.42	12.6	12.4	25 ± 1.36	6.2 ^b	1.25	1.54	0.44	1.34 ± 0.12	0.33 ^a	5.62 ± 0.31	0.38
	200	T ₂	2.04 ± 0.13		8.6	7.8	16.4 ± 1.09		0.87	1.5	0.51	1.07 ± 0.07		5.08 ± 0.38	
		C ₂	1.42 ± 0.23	0.62	12.4	12	24.4 ± 0.96	8 ^c	1.32	1.44	0.48	1.32 ± 0.11	0.25	5.68 ± 0.44	0.6
72	30	T ₁	2.28 ± 0.18		7.6	8.6	16.2 ± 0.89		0.7	1.23	0.45	0.89 ± 0.09		6.18 ± 0.10	
		C ₁	1.52 ± 0.10	0.76 ^b	10	11	21 ± 1.45	4.8 ^a	1.21	1.64	0.62	1.38 ± 0.04	0.49 ^c	7.02 ± 0.20	0.84 ^b
	200	T ₂	2.5 ± 0.20		5.8	6.6	12.4 ± 1.81		0.64	1.24	0.40	0.83 ± 0.13		6.16 ± 0.24	
		C ₂	1.86 ± 0.23	0.64	9.8	9.6	19.4 ± 1.48	7 ^b	1.5	1.16	0.59	1.38 ± 0.13	0.55 ^a	6.98 ± 0.30	0.82
96	30	T ₁	1.98 ± 0.20		6	9.2	15.2 ± 0.65		0.44	1.22	0.32	0.63 ± 0.05		3.62 ± 0.54	
		C ₁	1.64 ± 0.16	0.34	9.4	9	18.4 ± 1.44	3.2	1.01	1.11	0.51	1.03 ± 0.08	0.40 ^b	4.68 ± 0.60	1.06
	200	T ₂	2.2 ± 0.22		5	7	12 ± 2.44		0.39	0.95	0.33	0.53 ± 0.04		3.80 ± 0.29	
		C ₂	1.48 ± 0.10	0.72 ^a	8.2	8.2	16.6 ± 1.15	4.6	0.91	0.96	0.53	0.93 ± 0.11	0.40 ^b	5.12 ± 0.10	1.32 ^b

a = p < 0.05, b = p < 0.01, c = p < 0.001 (significance levels of t-test) Mitotic Indices (MI) in 5000 cells (1000 cells from each individuals), Micronuclei in normochromatic erythrocytes (NCE) and polychromatic erythrocytes (PCE) in 5000 cells (1000 cells from each individuals) and Sperm head-shape abnormality (SHA) in 5000 sperm (1000 sperm from each individuals) at different fixation intervals in CdCl₂ treated mice pre-fed with Cad Sulph-30 (T₁) and Cad Sulph-200 (T₂) separately and respective alcohol-30 (C₁) and alcohol-200 (C₂) fed controls.

control with unsuccessful dilute alcohol was also used to be maintained, but since no significant difference was observed in the results between successful alcohol fed series and dilute alcohol fed series, data of this control were not considered and incorporated in the present study for limiting the size of the tables.

One group (5 mice) of normal healthy mice were scanned for spontaneous levels of CA, MNE, MI and SHA, and another group (5 mice each), only fed with repeated doses of Cad sulph-30 or Cad sulph-200 at intervals corresponding to drug fed series, were also scanned for the same protocols.

Laboratory methodology

Chromosome aberration (CA) study

Chromosomes were prepared from the bone marrow cells by following the conventional colchicine-citrate-flame-drying-Giemsa stain method. The nomenclature

of Crippa [36] was followed in grouping the chromosomes into five size-classes.

Mitotic Index (MI) and Micronuclei (MNE) studies

For the study of MI and MNE, bone marrow cells of mice not treated with colchicine were centrifuged in 1% sodium citrate solution. The materials collected at the bottom were then smeared on clean grease free slides and allowed to air-dry. Air-dried slides were stained with May-Grunwald-Giemsa as per the conventional method [37,38].

Sperm head anomaly (SHA) study

For the study of SHA, sperm collected from epididymis in normal saline. It was made free from fat, vas deferens and other tissue debris. Then the material was thoroughly shaken to make the sperm free to suspend in the saline solution. The sperm suspension was filtered, spread on clean grease-free slides, air dried and stained with Giemsa as per the routine technique [39].

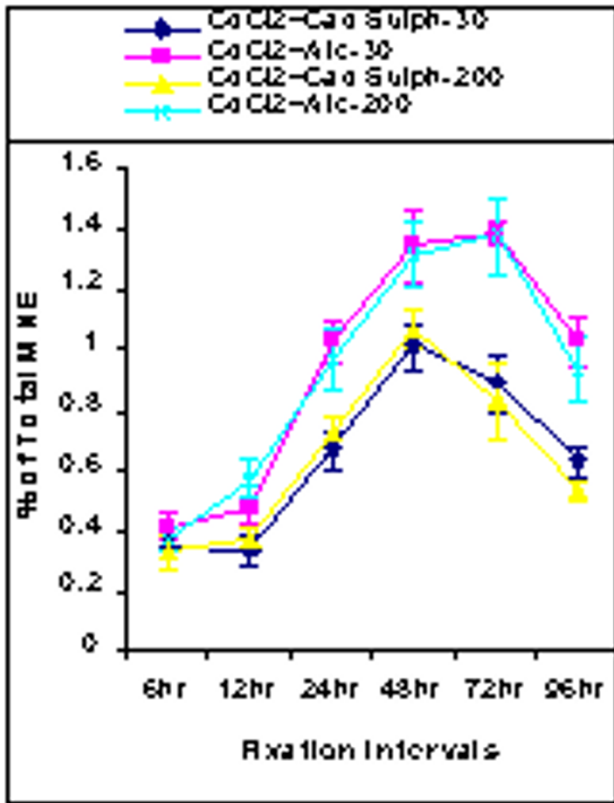


Figure 4 Showing time-dependent frequencies of Cad Sulph-30 pre-fed and Cad Sulph-200 pre-fed series versus their respective CdCl₂ treated alcohol-30 and alcohol-200 control mice. Chromosome aberration (CA)-1; Mitotic index (MI)-2; Micronucleated erythrocytes (MNE)-3 and Sperm head anomaly (SHA)-4.

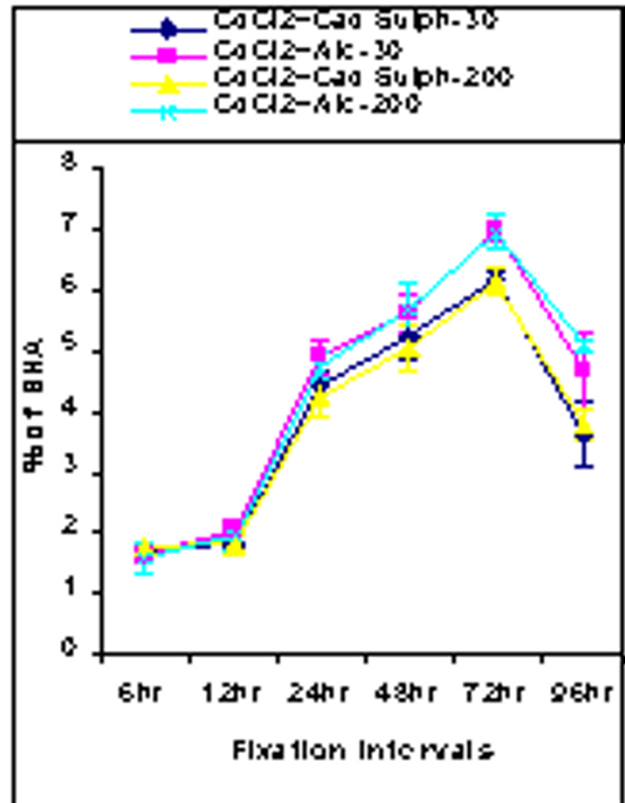


Figure 5 Showing time-dependent frequencies of Cad Sulph-30 pre-fed and Cad Sulph-200 pre-fed series versus their respective CdCl₂ treated alcohol-30 and alcohol-200 control mice. Chromosome aberration (CA)-1; Mitotic index (MI)-2; Micronucleated erythrocytes (MNE)-3 and Sperm head anomaly (SHA)-4.

Scoring of data

The observer was "blinded" during observation and scoring of different parameters in the treated and control series to remove the possibility of any 'bias' during scoring of data.

Statistical analysis

The test of significance between the data of the experimental and control series was determined by Student's t-test and the analysis of variance (two way ANOVA). The levels of significance of t-values were determined according to Fisher and Yates [40] statistical tables.

Results

Qualitative changes

A single injection of CdCl₂ induced various types of chromosome aberrations (CA) in bone marrow cells, a few representative types of which, namely, gap (Photomicrograph 1a), break (Photomicrograph 1b), fragment of un-

known origin (Photomicrograph 1c), terminal association (Photomicrograph 1d), ring and constriction (Photomicrograph 1e), precocious centromeric separation (Photomicrograph 1f), C-mitotic effect (Photomicrograph 1g), pulverisation (Photomicrograph 1h), erosion (Photomicrograph 1i) and polyploidy (Photomicrograph 1j) have been furnished. Micronucleated erythrocytes (MNE) – both polychromatic (Photomicrograph 1k) and normochromatic (Photomicrograph 1l) were also found in certain bone marrow cells. Sperm with abnormal head-shapes (Sperm head anomaly-SHA) (Photomicrograph 1m to 1o) were also recorded.

Quantitative changes

The frequency distributions of CA, MNE, MI and SHA in all the drug-treated series vis-a-vis control series were analysed. In both the normal healthy mice and mice fed with Cad Sulph-30 and 200 in repeated doses, the frequencies of CA, MNE, abnormal sperm shapes and MI

remained more or less within the spontaneous levels. Therefore, only the positive control (alcohol-30 or alcohol-200) was considered for comparison with the drug fed series for the sake of limiting the size of the tables and space.

Chromosome aberrations (CA) study

Drug pre-fed series

Both Cad Sulph-30 and Cad Sulph-200 could reduce the frequencies of CA in almost all the fixation intervals except for Cad Sulph-30 at 6 hr and the reductions were quite significant in later intervals ($p < 0.05 - p < 0.001$). The maximum protection was shown at 48 hr in case of both the drugs after which the extent of protection declined. When the efficacy of two potencies was compared Cad Sulph-200 showed more protection in all the fixation intervals than that of Cad Sulph-30 (Table 1, Fig. 2).

Drug post-fed series

Both Cad Sulph-30 and Cad Sulph-200 could reduce the frequencies of CA in all the fixation intervals. Both the drugs showed maximum protection at 24 hr, after which it declined. Again Cad Sulph-200 showed more protection in all the fixation intervals than that of Cad Sulph-30 (Table 2, Fig. 6).

Combined pre- and post-drug-fed series

Both the drugs showed significant (except for 6 hr) protections in all the fixation intervals. Cad Sulph-200 showed more protection than that of Cad Sulph-30 (Table 3, Fig. 10).

When the modes of treatment were compared in respect of CA it was found that post treatment was more effective than that of pre-treatment and the combined pre and post treatment was the most effective as compared to the others.

Table 2: Frequency distribution of Chromosome Aberrations (CA) in 500 bone marrow cells examined (100 cells from each individuals).

Intervals (hr)	Drug potency	Series	Mitotic Index		Chromosome Aberration				Micronucleated Erythrocyte					Sperm Head Anomaly	
			% ± SE	% of Increased MI	% of Major CA	% of Other CA	Total CA ± SE	Prot.	% of MN in NCE	% of MN in PCE	P/N	% of Total MN in NCE & PCE	% of Prot.	% of SHA ± SE	% of prot.
6	30	T ₃	1.72 ± 0.18		3.2	6	9.2 ± 0.82		0.15	1.07	0.12	0.25 ± 0.04		1.62 ± 0.20	
		C ₃	1.84 ± 0.20	-0.12	3.8	7.4	11.2 ± 0.96	2.0	0.23	0.73	0.32	0.35 ± 0.02	0.10 ^a	1.68 ± 0.25	0.06
	200	T ₄	1.98 ± 0.23		3.4	5.2	8.6 ± 1.20		0.2	0.67	0.13	0.25 ± 0.04		1.48 ± 0.19	
		C ₄	1.6 ± 0.18	0.38	5	6.6	11.6 ± 1.44	3.0	0.23	0.51	0.30	0.29 ± 0.03	0.04 ^a	1.56 ± 0.18	0.08
12	30	T ₃	1.84 ± 0.14		6	6.8	12.8 ± 0.82		0.2	0.58	0.25	0.27 ± 0.04		1.64 ± 0.18	
		C ₃	1.36 ± 0.17	0.48	7.6	9.8	17.4 ± 1.44	4.6 ^a	0.43	0.64	0.45	0.49 ± 0.05	0.22 ^a	2.12 ± 0.26	0.48
	200	T ₄	2.08 ± 0.14		4.8	4.8	9.6 ± 1.03		0.17	0.41	0.23	0.21 ± 0.02		1.32 ± 0.16	
		C ₄	1.38 ± 0.19	0.7 ^a	6.8	9.8	16.6 ± 1.60	7.0 ^a	0.4	0.57	0.45	0.47 ± 0.06	0.26 ^a	2.04 ± 0.23	0.72 ^a
24	30	t ₃	1.7 ± 0.33		8.2	9.4	17.6 ± 1.52		0.54	1	0.31	0.65 ± 0.05		3.6 ± 0.24	
		C ₃	1.1 ± 0.21	0.6	15.2	14.6	29.8 ± 1.55	12.2 ^c	0.81	1.44	0.28	0.95 ± 0.08	0.30 ^a	4.98 ± 0.28	1.38 ^b
	200	T ₄	1.88 ± 0.35		6.6	6.6	13.2 ± 2.07		0.36	0.62	0.40	0.43 ± 0.04		3.1 ± 0.24	
		C ₄	0.98 ± 0.22	0.9	16.4	14.2	30.6 ± 1.56	17.4 ^c	0.86	1.69	0.28	1.05 ± 0.07	0.62 ^c	5.12 ± 0.28	2.02 ^c
48	30	t ₃	2 ± 0.16		7.8	9	16.8 ± 0.77		0.54	0.76	0.45	0.61 ± 0.08		4.56 ± 0.39	
		C ₃	0.98 ± 0.14	1.02 ^b	11	12.8	23.8 ± 1.34	7.0 ^b	1.34	1.28	0.54	1.32 ± 0.08	0.71 ^c	5.9 ± 0.41	1.34 ^a
	200	T ₄	2.34 ± 0.16		7	7.4	14.4 ± 0.90		0.32	0.41	0.50	0.35 ± 0.02		4.06 ± 0.39	
		C ₄	1.06 ± 0.13	1.28 ^c	12	13.8	25.8 ± 0.96	11.4 ^c	1.15	1.41	0.50	1.22 ± 0.04	0.87 ^c	6.02 ± 0.38	1.96 ^b
72	30	t ₃	2.76 ± 0.21		5.6	7.2	12.8 ± 1.38		0.47	0.64	0.33	0.51 ± 0.06		7.64 ± 0.16	
		C ₃	1.7 ± 0.18	1.06 ^b	9.6	10.4	20 ± 1.45	7.2 ^b	1.65	1.06	0.58	1.44 ± 0.09	0.93 ^c	7.74 ± 0.30	0.10
	200	T ₄	2.9 ± 0.22		6.6	4.4	11 ± 1.50		0.26	0.59	0.30	0.33 ± 0.05		4.86 ± 0.33	
		C ₄	1.56 ± 0.07	1.34 ^c	9.4	11.2	20.6 ± 1.64	9.6 ^c	1.17	1.62	0.60	1.32 ± 0.08	0.99 ^c	7.28 ± 0.20	2.42 ^c
96	30	t ₃	2.3 ± 0.23		4.6	7.6	12.2 ± 1.14		0.28	0.92	0.17	0.37 ± 0.06		2.72 ± 0.18	
		C ₃	1.26 ± 0.15	1.04 ^c	8.2	10.2	18.4 ± 1.60	6.2 ^a	0.86	0.94	0.49	0.91 ± 0.09	0.54 ^c	4.64 ± 0.34	1.92 ^b
	200	T ₄	2.56 ± 0.26		3.2	5.6	9.8 ± 1.24		0.18	0.71	0.16	0.25 ± 0.02		2.38 ± 0.24	
		C ₄	1.42 ± 0.10	1.14 ^b	7.4	10.4	17.6 ± 1.60	7.8 ^b	0.94	1.01	0.49	0.97 ± 0.09	0.72 ^c	5.14 ± 0.19	2.76 ^c

a = $p < 0.05$, b = $p < 0.01$, c = $p < 0.001$ (significance levels of t-test) Mitotic Indices (MI) in 5000 cells (1000 cells from each individuals), Micronuclei in normochromatic erythrocytes (NCE) and polychromatic erythrocytes (PCE) in 5000 cells (1000 cells from each individuals) and Sperm head-shape abnormality (SHA) in 5000 sperm (1000 sperm from each individuals) at different fixation intervals in CdCl₂ treated mice post-fed with Cad Sulph-30 (T₃) and Cad Sulph-200 (T₄) separately and respective alcohol-30 (C₃) and alcohol-200 (C₄) fed controls.

Table 3: Frequency distribution of Chromosome Aberrations (CA) in 500 bone marrow cells examined (100 cells from each individuals).

Intervals (hr)	Drug potency	Mitotic Index		Chromosome Aberration				Micronucleated Erythrocyte					Sperm Head Anomaly		
		% ± SE	% of Increased MI.	% of Major CA	% of Other CA	% of Total CA ± SE	% of Prot.	% of MN in NCE	% of MN in PCE	P/N	% of Total MN in NCE & PCE	% of Prot.	% of SHA ± SE	% of prot.	
6	30	T ₅	1.84 ± 0.22		3.4	5.4	8.8 ± 1.14		0.15	0.58	0.11	0.21 ± 0.02		1.6 ± 0.15	
		C ₅	1.42 ± 0.14	0.42	4.4	6	10.4 ± 1.82	1.6	0.31	0.72	0.33	0.41 ± 0.04	0.20 ^a	1.64 ± 0.07	0.04
	200	T ₆	2.1 ± 0.25		2	3	5 ± 1.17		0.14	0.7	0.24	0.22 ± 0.05		1.72 ± 0.17	
		C ₆	1.48 ± 0.16	0.62	4.2	4.6	10.8 ± 1.14	5.8	0.33	1.06	0.51	0.48 ± 0.05	0.26 ^b	1.5 ± 0.12	-0.22
12	30	T ₅	2.24 ± 0.12		4.8	6.4	11.2 ± 0.65		0.16	0.59	0.20	0.27 ± 0.02		1.7 ± 0.22	
		C ₅	1.56 ± 0.13	0.68 ^b	8	9.4	17.4 ± 1.68	6.2 ^b	0.43	0.77	0.49	0.55 ± 0.05	0.32 ^c	2.16 ± 0.28	0.46 ^a
	200	T ₆	2.42 ± 0.12		3	4.8	7.4 ± 0.74		0.14	0.38	0.18	0.17 ± 0.02		1.52 ± 0.19	
		C ₆	1.46 ± 0.13	0.96 ^c	5.8	10.2	16 ± 1.54	8.2 ^c	0.36	0.81	0.45	0.51 ± 0.04	0.34 ^c	2.12 ± 0.21	0.6
24	30	T ₅	2.04 ± 0.18		6.0	6.6	12.6 ± 1.2		0.31	1	0.31	0.47 ± 0.04		3.02 ± 0.19	
		C ₅	1.1 ± 0.20	0.94 ^b	14.6	15.6	30.2 ± 1.63	17.6 ^c	0.91	1.81	0.32	1.12 ± 0.08	0.77 ^c	5.09 ± 0.30	2.07 ^c
	200	T ₆	2.28 ± 0.26		4.8	5.6	10.4 ± 1.30		0.18	0.47	0.32	0.25 ± 0.02		2.64 ± 0.23	
		C ₆	0.98 ± 0.17	1.3 ^b	13.4	18.4	31.8 ± 1.55	21.4 ^c	0.87	1.72	0.30	1.05 ± 0.10	0.80 ^c	4.96 ± 0.57	2.32 ^b
48	30	T ₅	2.44 ± 0.09		5.6	5.8	11.4 ± 1.03		0.36	0.53	0.51	0.41 ± 0.06		3.64 ± 0.29	
		C ₅	1.44 ± 0.20	1 ^b	12.4	13.6	26 ± 1.87	14.6 ^c	0.98	1.63	0.46	1.20 ± 0.04	0.79	5.64 ± 0.34	2 ^b
	200	T ₆	2.84 ± 0.16		5.0	5.8	10.8 ± 1.14		0.12	0.44	0.55	0.23 ± 0.02		3.38 ± 0.35	
		C ₆	1.16 ± 0.13	1.68 ^c	13.0	14	27 ± 1.11	16.2 ^c	1.06	1.56	0.49	1.22 ± 0.05	0.99 ^c	5.74 ± 0.32	2.36 ^b
72	30	T ₅	2.86 ± 0.14		3.2	5.6	8.8 ± 1.14		0.7	1.23	0.46	0.89 ± 0.09		4.72 ± 0.23	
		C ₅	1.68 ± 0.09	1.18 ^c	8.0	9.4	17.4 ± 1.51	8.6 ^c	1.21	1.64	0.57	1.38 ± 0.04	0.49 ^c	7.06 ± 0.24	2.34 ^c
	200	T ₆	3.18 ± 0.28		3.6	4.8	8.4 ± 1.33		0.12	0.55	0.27	0.21 ± 0.04		5.38 ± 0.20	
		C ₆	1.6 ± 0.09	1.58 ^c	9.0	12.4	21.4 ± 1.30	13 ^c	1.45	0.64	0.64	1.38 ± 0.09	1.17 ^c	7.96 ± 0.35	2.58 ^c
96	30	T ₅	2.68 ± 0.24		3.8	4.8	8.6 ± 0.90		0.27	0.69	0.29	0.37 ± 0.02		2.18 ± 0.15	
		C ₅	1.68 ± 0.13	1.00 ^b	9.2	10	19.2 ± 1.55	10.6 ^c	1.18	1.34	0.46	1.20 ± 0.04	0.83 ^c	4.88 ± 0.30	2.7 ^c
	200	T ₆	3 ± 0.23		3.6	4.4	8 ± 0.61		0.15	0.29	0.29	0.23 ± 0.02		1.86 ± 0.13	
		C ₆	1.58 ± 0.13	1.42 ^b	9.0	9.8	18.8 ± 1.31	10.8 ^c	1.06	0.45	0.45	1.10 ± 0.07	0.87 ^c	5.02 ± 0.14	3.16 ^c

a = p < 0.05, b = p < 0.01, c = p < 0.001 (significance levels of t-test) Mitotic Indices (MI) in 5000 cells (1000 cells from each individuals), Micronuclei in normochromatic erythrocytes (NCE) and polychromatic erythrocytes (PCE) in 5000 cells (1000 cells from each individuals) and Sperm head-shape abnormality (SHA) in 5000 sperm (1000 sperm from each individuals) at different fixation intervals in CdCl₂ treated mice combined pre- and post-fed with Cad Sulph-30 (T₅) and Cad Sulph-200 (T₆) separately and respective alcohol-30 (C₅) and alcohol-200 (C₆) fed controls.

Mitotic Index (MI) study

Drug pre-fed series

Both Cad Sulph-30 and Cad Sulph-200 could enhance the frequencies of MI in all the fixation intervals and the maximum enhancement was shown at the later intervals (24–96 hr). When the efficacy of two drugs were compared Cad Sulph-200 showed more enhancement of MI than that of Cad Sulph-30 in almost all the fixation intervals except for 72 hr where it was just the opposite (Table 1, Fig. 3).

Drug post-fed series

Both the drugs could enhance the frequencies of MI in all the fixation intervals and the maximum enhancement was shown at 72 hr (p < 0.01 in case of Cad sulph-30 and p < 0.001 in case of Cad sulph-200). Again when the efficacy of two drugs were compared Cad Sulph-200 showed more enhancement of MI than that of Cad Sulph-30 in all the fixation intervals (Table 2, Fig. 7).

Combined pre and post drug-fed series

Both the drugs showed increase in MI at all the fixation intervals and those were statistically significant except for 6 hr. Further, Cad Sulph-200 increased MI more than that of Cad Sulph-30 (Table 3, Fig. 11).

When the modes of treatment were compared in respect of MI, it was found that post treatment was more effective than that of pre-treatment and the combined pre- and post-treatment was the most effective.

Micronuclei (MN) study

Drug pre-fed series

Both Cad Sulph-30 and Cad Sulph-200 could reduce the frequencies of NCE, PCE and total MNE in all the fixation intervals. The maximum protection was shown at 24 hr in case of both the drugs and after that it declined. When the efficacy of two drugs was compared Cad Sulph-200 showed more protection in all the fixation intervals than that of Cad Sulph-30 (Table 1, Fig. 4).

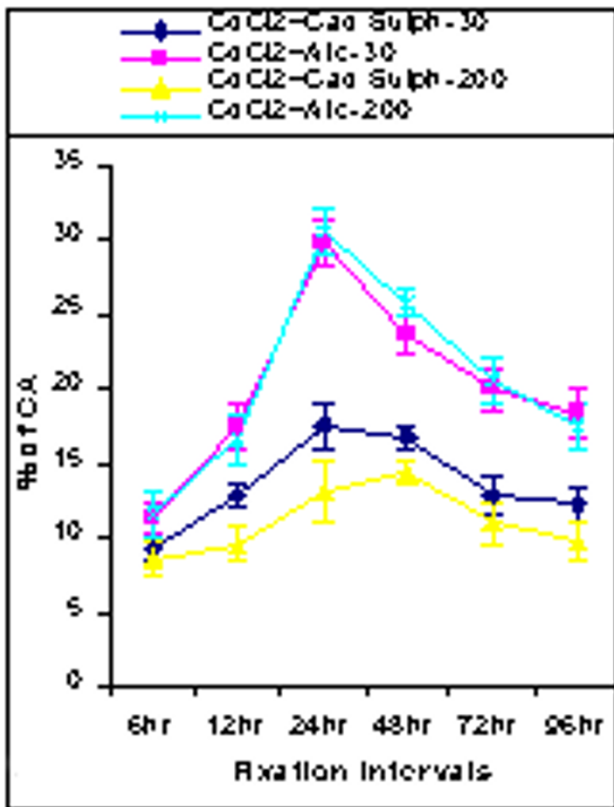


Figure 6 Showing time-dependent frequencies of Cad Sulph-30 post-fed and Cad Sulph-200 post-fed series versus their respective CdCl₂ treated alcohol-30 and alcohol-200 control mice. Chromosome aberration (CA)-5; Mitotic index (MI)-6; Micronucleated erythrocytes (MNE)-7 and Sperm head anomaly (SHA)-8.

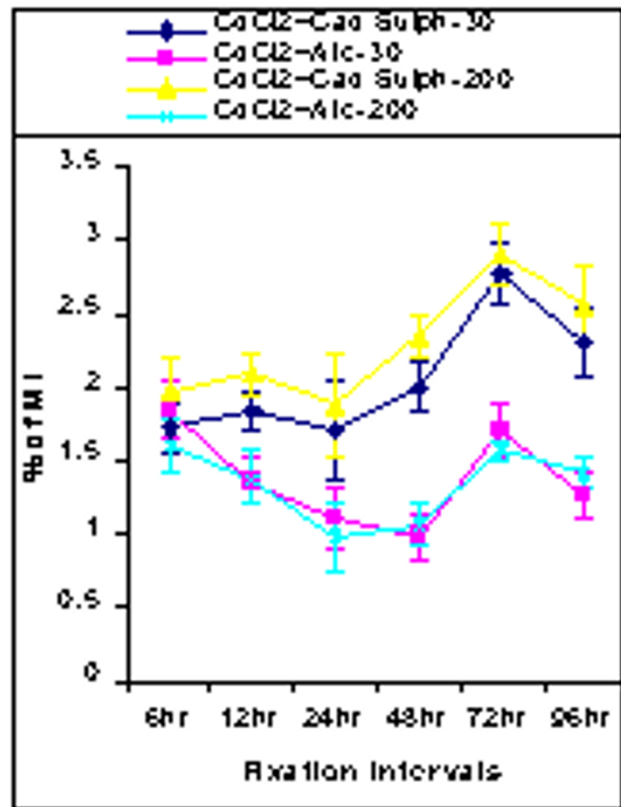


Figure 7 Showing time-dependent frequencies of Cad Sulph-30 post-fed and Cad Sulph-200 post-fed series versus their respective CdCl₂ treated alcohol-30 and alcohol-200 control mice. Chromosome aberration (CA)-5; Mitotic index (MI)-6; Micronucleated erythrocytes (MNE)-7 and Sperm head anomaly (SHA)-8.

Drug post-fed series

Both the drugs could reduce the frequencies of NCE, PCE and total MNE in all the fixation intervals and the differences were statistically significant in almost all the fixation intervals (except for Cad Sulph-200 at 6 hr). Cad Sulph-200 showed more protective ability than that of Cad Sulph-30 (Table 2, Fig. 8).

Combined pre and post drug-fed series

Both the potencies showed remarkable efficiency in reducing the frequencies of NCE, PCE and total MNE in all the fixation intervals and the differences were statistically highly significant ($p < 0.001$). Cad Sulph-200 showed more protection than Cad Sulph-30 (Table 3, Fig. 12).

When the modes of treatment were compared it was noticed that both the drugs could show more protection in combined pre- and post-feeding followed by only post-feeding.

Sperm head anomaly (SHA) study

Drug pre-fed series

Both Cad Sulph-30 and 200 could reduce the frequencies of sperm with abnormal head shapes in almost all the fixation intervals except for 6 hr and the 200th potency showed more protection than that of the 30th potency (Table 1, Fig. 5).

Drug post-fed series

Both the potencies showed reductions in frequencies of sperm with abnormal head shapes in all the fixation intervals and the differences were statistically significant in almost all the fixation intervals except for 6 hr. Cad Sulph-200 showed more protective ability than that of Cad Sulph-30 (Table 2, Fig. 6).

Combined pre and post drug-fed series

The same trend was noticed for both potencies of the drug (except for Cad Sulph-200 at 6 hr) (Table 3, Fig. 13).

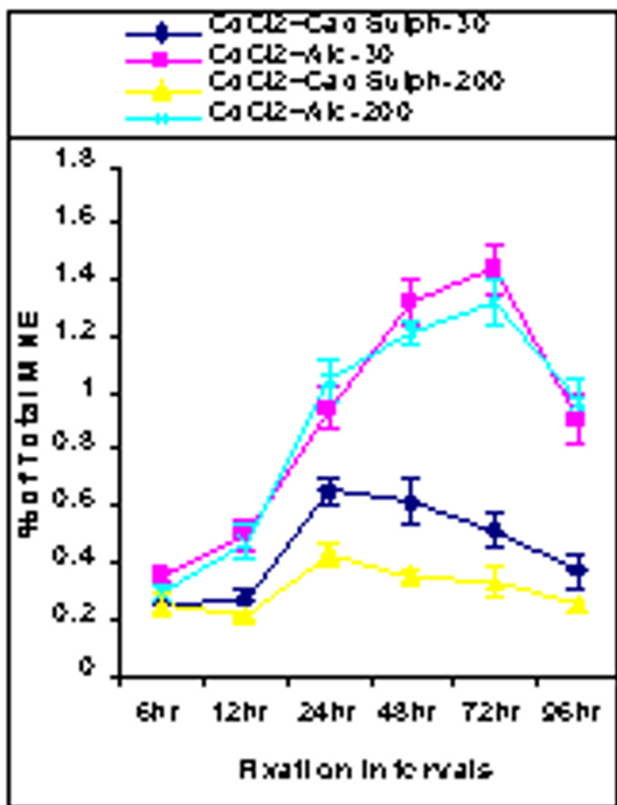


Figure 8
Showing time-dependent frequencies of Cad Sulph-30 post-fed and Cad Sulph-200 post-fed series versus their respective CdCl₂ treated alcohol-30 and alcohol-200 control mice. Chromosome aberration (CA)-5; Mitotic index (MI)-6; Micronucleated erythrocytes (MNE)-7 and Sperm head anomaly (SHA)-8.

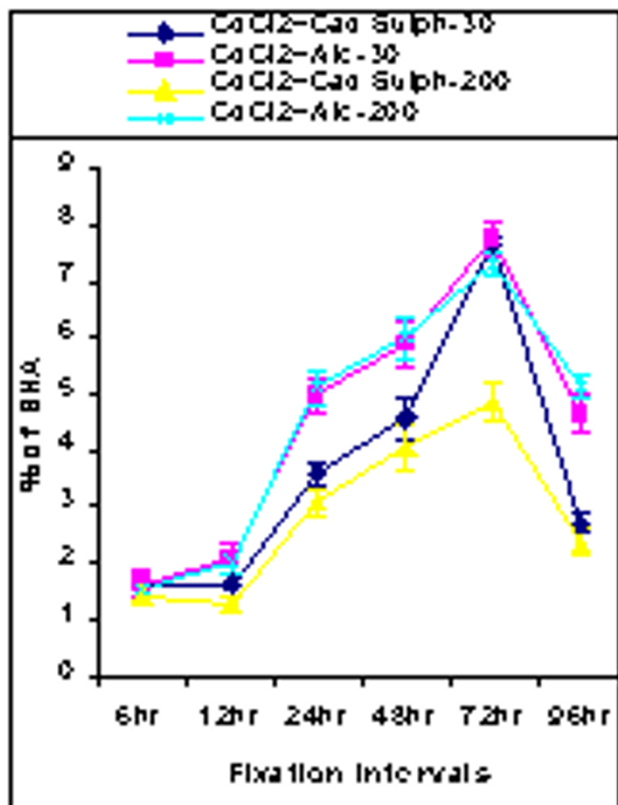


Figure 9
Showing time-dependent frequencies of Cad Sulph-30 post-fed and Cad Sulph-200 post-fed series versus their respective CdCl₂ treated alcohol-30 and alcohol-200 control mice. Chromosome aberration (CA)-5; Mitotic index (MI)-6; Micronucleated erythrocytes (MNE)-7 and Sperm head anomaly (SHA)-8.

The maximum protective ability of both potencies of the drug was noticed in combined pre- and post-feeding followed by post-and pre-feeding.

Discussion

From the results of the present investigation it is revealed that both Cad Sulph-30 and Cad Sulph-200 showed remarkable potential to reduce genotoxic effects produced by CdCl₂ although the drug itself had no palpable genotoxic effects of its own when fed to normal healthy mice. Incidentally the results were essentially similar to different potencies of Ars Alb used against arsenic poisoning [30-35,41,42]. It would also be revealed clearly that Cad sulph-200 rendered more pronounced protective effect, particularly at longer fixation intervals.

Cadmium poisoning in certain areas, particularly at or near Zinc mines and Zinc processing factories, paint industries, has crossed tolerable limits. Incidentally, cad-

mium has been reported to significantly inhibit *E. coli* and human DNA polymerase activity [43,44]. When growing cultures of *E. coli* were exposed to cadmium, considerable single-strand breaks were reported to occur in DNA [45]. Further, Privezentsev *et al*[46] reported that cadmium genotoxicity observed in both *in vivo* and *in vitro* cells of mice would be due to single-strand breaks in DNA through the direct cadmium-DNA interactions. In our present study also, we noted quite a good number of plates with clear chromosomal damage indicative of DNA breaks and other forms of lesions. Therefore, the search for a protective agent which may be used without any fear or risk of toxic side effects to prevent/repair such damages seemed to us to be important. From the encouraging and positive modulating action of the potentized Cad Sulph observed in the present study, these drugs could be suggested as strong candidates for combating unintentional/accidental exposure to sub-

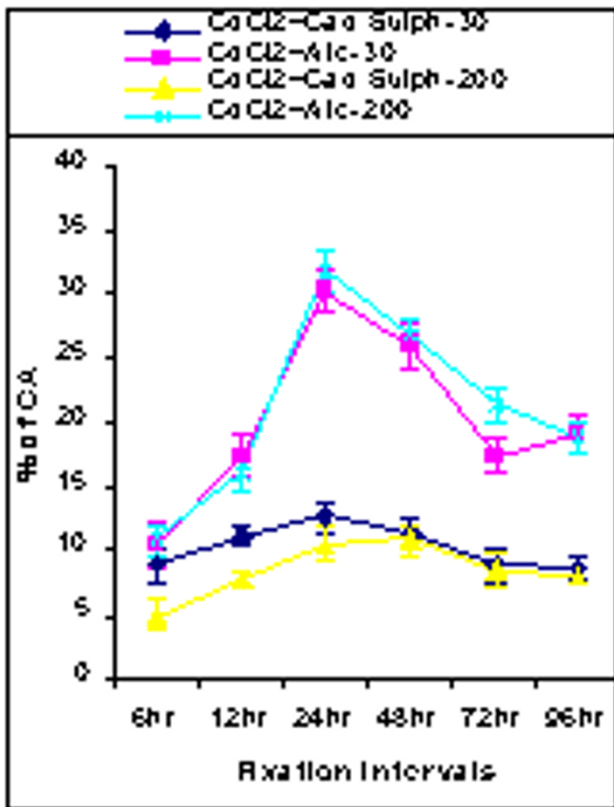


Figure 10
Showing time-dependent frequencies of Cad Sulph-30 combined pre- and post-fed and Cad Sulph-200 combined pre- and post-fed series versus their respective CdCl₂ treated alcohol-30 and alcohol-200 control mice. Chromosome aberration (CA)-9; Mitotic index (MI)-10; Micronucleated erythrocytes (MNE)-11 and Sperm head anomaly (SHA)-12.

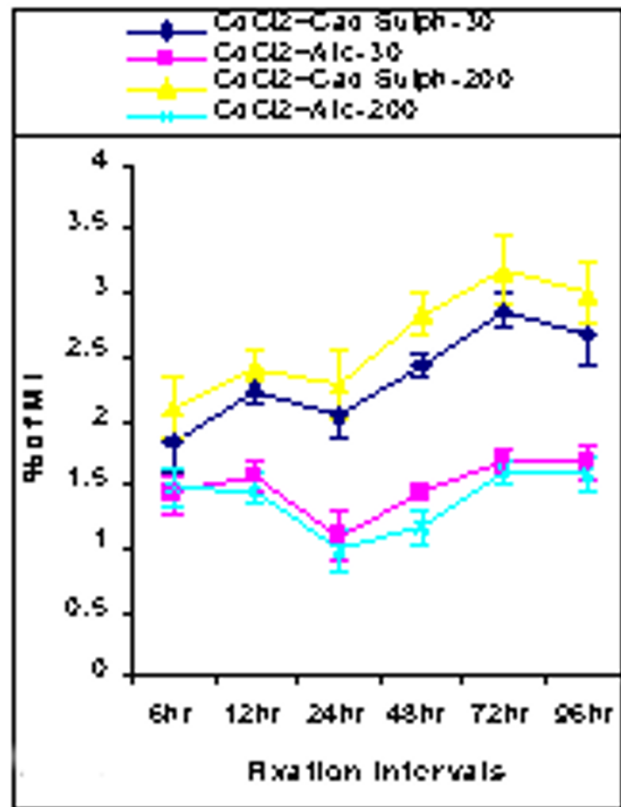


Figure 11
Showing time-dependent frequencies of Cad Sulph-30 combined pre- and post-fed and Cad Sulph-200 combined pre- and post-fed series versus their respective CdCl₂ treated alcohol-30 and alcohol-200 control mice. Chromosome aberration (CA)-9; Mitotic index (MI)-10; Micronucleated erythrocytes (MNE)-11 and Sperm head anomaly (SHA)-12.

acute cadmium poisoning, both as a preventive as well as curative agent.

In the present study the homeopathic drug apparently enhanced/activated the process of maintaining the structural integrity of chromosomes and sperm either protecting them from the destructive ability of CdCl₂ in causing DNA damage or else, by enhancing the process of repair of DNA already damaged by activating specific enzyme systems to repair the damage.

Incidentally, there are inherent mechanisms of DNA and chromosome repairs [47,48] which are known to be genetically controlled. The same is true for the regulation of cell cycle events. In the present study also we have noted a positive shift in the mitotic index in the CdCl₂-fed mice which may be linked to the replenishment of bone marrow cells, the necessity arising out of the exclusion/loss of damaged marrow cells due to the toxic chemical inter-

action of CdCl₂ to chromosome components primarily made up of DNA and protein. The repair mechanisms of chromosomes, therefore, also involved inherent mechanisms for repair of protein damage, a process also under a precise genetical control [47-49]. Therefore, any mechanism that can repair/protect cytogenetical effects must necessarily be regulated by specific genetic mechanisms essentially controlled by specific genes. Theoretically speaking even in the absence of a single original drug molecule both Cad Sulph-30 and 200 elicited spectacular ability of protection/repair to damaged chromosomes and sperm, a fact which would lead one to speculate that the drugs must have acted through the genetic regulatory mechanisms. In fact, Khuda-Bukhsh [50] explained the possible mechanism of action of the potentized homeopathic drugs by suggesting that one major way by which the potentized homeopathic drugs acted was possibly through regulation of expression of certain genes. This could be achieved by activating cer-

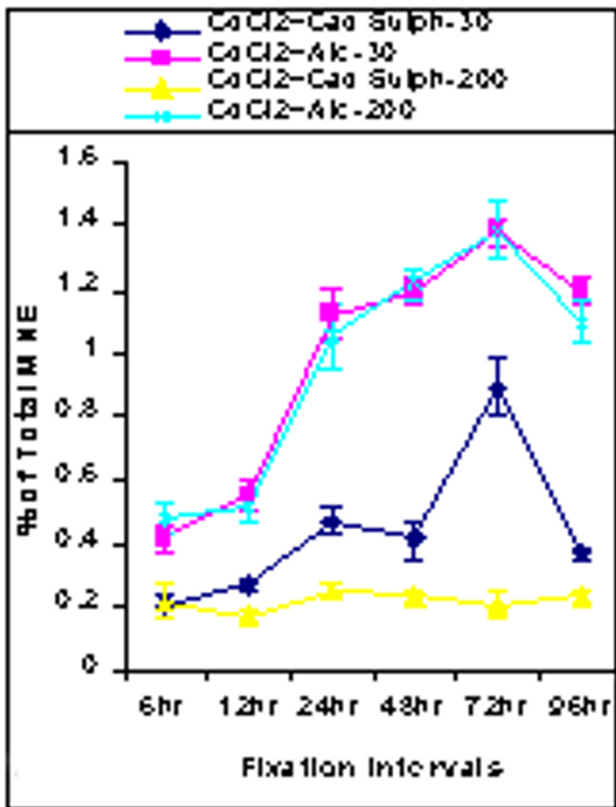


Figure 12 Showing time-dependent frequencies of Cad Sulph-30 combined pre- and post-fed and Cad Sulph-200 combined pre- and post-fed series versus their respective CdCl₂ treated alcohol-30 and alcohol-200 control mice. Chromosome aberration (CA)-9; Mitotic index (MI)-10; Micronucleated erythrocytes (MNE)-11 and Sperm head anomaly (SHA)-12.

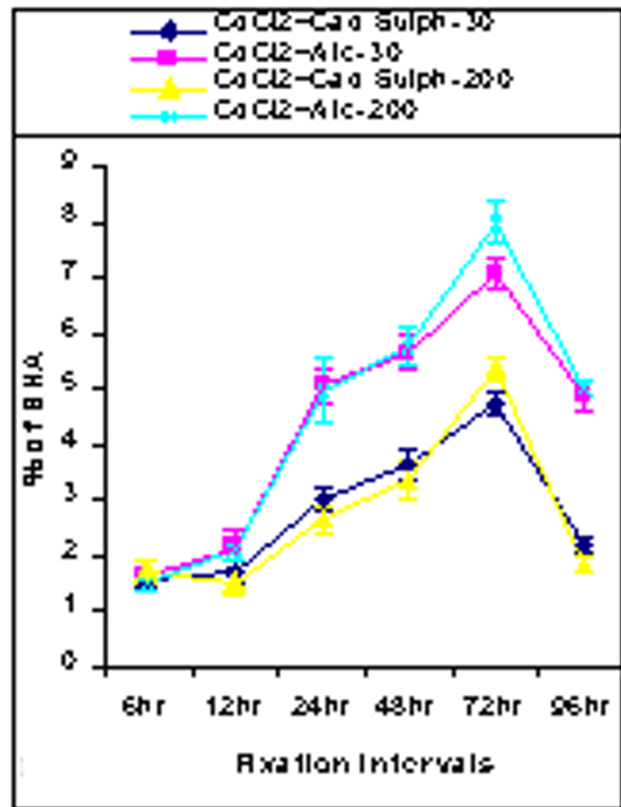


Figure 13 Showing time-dependent frequencies of Cad Sulph-30 combined pre- and post-fed and Cad Sulph-200 combined pre- and post-fed series versus their respective CdCl₂ treated alcohol-30 and alcohol-200 control mice. Chromosome aberration (CA)-9; Mitotic index (MI)-10; Micronucleated erythrocytes (MNE)-11 and Sperm head anomaly (SHA)-12.

tain hormones and enzymes through transduction of specific regulatory signals including transcription factors (either inducible or repressive in nature) on target cells, in order to get them back to their normal state of functioning. The results of this study will also strengthen this view.

Conclusion

In conclusion, it may be pointed out that the two microdoses of Cad Sulph were capable of rendering protection/repair to the genotoxic damages caused by the treatment of the toxic chemical, CdCl₂, and these were most effective when the combined pre- and post-feeding mode was followed. However, only post-feeding also proved to be effective in restoring the damages to a considerable extent. Interesting to note, however, is the fact that the more diluted, or the 200th potency appeared to have more pronounced efficacy in rendering protective effects than that of the 30th potency, as is claimed in the

homeopathic literature. Since there is also some amount of protective effects of Cad Sulph against CdCl₂ poisoning, and since combined pre- and post-feeding mode appeared to show the maximum protective effect, and since Cad Sulph itself had no damaging effects of its own on the genome, the preventive administration of Cad Sulph to people living in contaminated zone may be considered.

Acknowledgement

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