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Toxicological Screening of a Trace Element, Manganese *In vivo*

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Manganese, a metal of the first group of transitional element is essential for nutrition in higher organisms (Joardar *et al.* 1988, WHO 1981). The metal has been found to be the least toxic of essential elements though excess leads to poisoning and different degrees of damage to the exposed organs (Venugopal and Luckey 1978).

Manganese toxicity is one of the most important factor causing growth limitation in acid soils (Foy 1983, 1984) and in its different forms it is also a clastogenic and cytotoxic for higher organisms (Joardar 1988, Shukla and Singhal 1984, WHO 1981). Information on the effects of Mn on cell division and chromosomes is relatively meagre. In the present study two different plant systems and one mammalian system were used for evaluation of the cytotoxicity and clastogenicity of the trace element, manganese.

Materials and methods

a) *Test chemical*

The test chemical used was the soluble salt of manganese, manganese sulphate, $MnSO_4 \cdot H_2O$ (Sarabhai Chemicals, Bombay).

b) *Plant test systems*

Equal sized bulbs from a population of a commercial variety of the garlic, *Allium sativum* L. (2n=16) and seeds of *Pisum sativum* L. (2n=14) were used as plant test materials.

c) *Animal test system*

Laboratory bred Swiss albino mice, *Mus musculus* L. was used as animal test system.

d) *Doses used*

For plant test systems a concentration interval of 10^{-2} to 10^{-6}M of the test chemicals was used.

For mouse five different doses—102, 153, 203, 305 and 610 mg/kg body weight of the chemical were chosen.

e) *Duration of treatment*

For *Allium sativum* duration of treatment period was 168 hours and that for *Pisum sativum* was for 24 hours only. Mice were treated for 21 days.

f) *Protocol followed*

At regular intervals of 24 hours till 168 hours meristematic assays were carried out from root tips of both *Allium* and *Pisum* sp. Control sets were maintained for both the test systems. Slides were prepared following usual acetic-orcein squash method (Grant 1982, Sharma and Sharma 1980).

For all statistical analysis for plants the level of significance was established at an alpha of 0.05. Student's 't' tests were employed to compare the effects of the respective doses with the negative control.

For animal experiment 8 to 10 weeks of *Mus musculus* weighing about 25 to 30 g were

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taken. Five doses of $MnSO_4$ were administered orally in volumes of 0.1 ml/10 g body weight. Concurrent negative controls received only glass distilled water, the dilutent of the test chemical.

The animals were sacrificed on 7, 14 and 21 day and slides were prepared following usual flame drying technique of Preston *et al.* (1987), Sharma and Talukder (1974) and Sharma *et al.* (1983). To score aberrations, 75 good metaphase spreads were screened per animal (5 in a group).

A one tailed trend test (Margolin *et al.* 1986) was used to determine the dose related response, if any.

Table 1. Frequency of chromosomal aberrations in *Allium sativum* L. treated with $MnSO_4$

Treatment time (h)	Dose					
	C	D_1	D_2	D_3	D_4	D_5
24	0.58±0.21	3.41±0.89[a]	4.01±0.99[a]	7.28±1.35[a]	7.69±1.24[a]	8.51±2.30[a]
48	0.63±0.07	3.53±0.57[a]	4.27±1.05[a]	7.14±2.08[a]	9.00±4.83[b]	10.69±2.11[a]
72	0.66±0.08	4.02±0.77[a]	4.25±0.81[a]	8.28±1.19[c]	9.54±2.26[a]	11.36±1.51[a]
96	0.85±0.07	4.14±0.82[a]	4.98±1.10[a]	8.59±4.95[b]	9.28±2.17[b]	13.55±2.06[a]
120	0.85±0.05	4.27±1.09[b]	4.67±1.08[a]	8.91±3.60[b]	9.87±2.38[a]	—
144	0.89±0.03	4.29±0.97[a]	5.39±0.95[a]	8.94±2.84[a]	10.00±3.31[a]	—
168	1.48±0.26	6.00±1.54[a]	6.51±1.88[a]	8.99±1.21[a]	—	—

C=control, $D_1=6.6\times10^{-6}$, $D_2=6.6\times10^{-5}$, $D_3=6.6\times10^{-4}$, $D_4=6.6\times10^{-3}$, $D_5=6.6\times10^{-2}$.
Difference with untreated control value at the same fixing time is significant ([a]$P<0.001$, [b]$P<0.01$, [c]$P<0.05$), using student's 't' test.

Table 2. Frequency of chromosomal aberrations in *Pisum sativum* L. treated with $MnSO_4$

Treatment time (h)	Dose					
	C	D_1	D_2	D_3	D_4	D_5
24	0.97±0.06	2.24±1.51	4.37±1.87[b]	4.78±1.53[a]	5.13±1.27[a]	5.51±2.05[b]
48	1.01±0.05	2.16±1.29	3.47±1.39[b]	3.91±1.07[a]	5.04±2.01[b]	5.28±1.59[a]
72	1.00±0.06	1.45±0.53	3.09±1.08[b]	3.66±0.86[a]	3.93±1.24[a]	5.42±1.35[a]
96	0.99±0.05	1.26±0.38	3.04±1.05[b]	3.51±1.09[a]	3.83±1.08[a]	5.39±1.04[a]
120	1.02±0.04	1.65±0.85	2.93±1.85[c]	3.23±1.53[c]	3.69±2.02[c]	5.35±1.38[a]
144	0.94±0.12	1.33±1.09	2.91±1.08[b]	3.26±1.48[b]	3.78±1.27[b]	5.30±2.24[b]
168	0.95±0.11	1.09±0.84	2.87±0.98[b]	3.12±1.16[b]	3.32±0.88[a]	5.42±1.93[a]

C=control, $D_1=6.6\times10^{-6}$, $D_2=6.6\times10^{-5}$, $D_3=6.6\times10^{-4}$, $D_4=6.6\times10^{-3}$, $D_5=6.6\times10^{-2}$.
Difference with untreated control value at the same fixing time is significant ([a]$P<0.001$, [b]$P<0.01$, [c]$P<0.05$) using student's 't' test.

Observations

The cytotoxic and clastogenic effects of the salt exhibited a positive correlation with dose and period of treatment as shown by the high to highly significant level of increase in *Allium* sp. The most drastic effect was observed with 6.6×10^{-2}M (D_5). The types of abnormalities included both chromosome break, gap, fragments and spindle disturbances. Prolonged treatment with (D_5) 6.6×10^{-2}M caused root lethality (Table 1).

$MnSO_4$ showed a significant increase of abnormal cells in *Pisum* sp. As compared to break and fragments higher number of spindle disturbances were found in case of *Pisum* sp. (Table 2).

A comparison between two plant test systems indicated a significant difference with all doses. The frequency of abnormal cells gradually increased following treatment in *Allium*

but decreased in all cases except the highest dose in *Pisum* sp. (Table 3). All the doses of the salt induced high frequencies of abnormal cells with increased dose and duration of treatment. Breaks per cell showed a statistically significant trend for all the doses whereas trend test indicated a nonsignificant value for total chromosomal aberrations for 14 and 21 days treatment.

Discussion

Manganese showed a positive response in *in vitro* mammalian chromosome aberration and mutation (Hansen and Stern 1984). Assays with higher plants also showed positive res-

Table 3. Comparative effects of $MnSO_4$ on CA (chromosomal aberration) frequency of *Allium sativum* L. (A) and *Pisum sativum* L. (B)

Period of treatment (h)		Percentage of CA				
		D_1	D_2	D_3	D_4	D_5
24	A	3.41[c]	4.01	7.28[c]	7.69[c]	8.51
	B	2.24	4.37	4.78	5.13	5.51
72	A	4.02[a]	4.25	8.28[a]	9.54[a]	11.36[a]
	B	1.45	3.09	3.66	3.93	5.42
120	A	4.27[b]	4.67[b]	8.91[c]	9.87[b]	—
	B	1.65	2.93	3.23	3.69	5.35
168	A	6.00[a]	6.51[b]	8.99[a]	—	—
	B	1.09	2.87	3.12	3.32	5.42

$D_1=6.6\times10^{-6}$, $D_2=6.6\times10^{-5}$, $D_3=6.6\times10^{-4}$, $D_4=6.6\times10^{-3}$, $D_5=6.6\times10^{-2}$.
Difference between A and B at the same fixing time is significant ([a]$P<0.001$, [b]$P<0.01$, [c]$P<0.05$) using student's 't' test.

Table 4. Frequency of chromosomal aberrations (CA) and break per cells (B/C) following treatment with $MnSO_4$ in *Mus musculus* L.

Concentration of salt (in mg/kg b.w.)	Days after treatment					
	7 day		14 day		21 day	
	CA	B/C	CA	B/C	CA	B/C
0	8.46±0.83	1.14±0.05	9.28±0.36	0.64±0.06	12.15±0.39	0.61±0.05
102	19.53±1.45	4.98±0.83	19.25±0.57	5.18±0.67	24.49±2.73	5.23±0.33
153	30.04±3.31	5.01±0.79	35.09±2.05	4.54±1.20	32.65±3.66	3.60±0.29
203	38.05±1.84	12.15±1.41	44.13±2.22	12.98±3.12	46.41±0.60	10.73±0.94
305	41.28±5.51	12.98±1.03	50.93±2.58	10.31±0.84	47.09±2.20	12.86±0.65
610	49.93±4.04	15.46±1.45	51.93±5.28	15.19±0.47	53.39±3.38	14.48±0.33
Trend test P value	0.0001*	0.0001*	0.2676	0.0001*	0.4483	0.0001*

* Significant value at $\alpha=0.05$ level by a one-tailed trend test.

ponse. Kennedy and Bryant (1986) and Kosyanenko *et al.* (1987) reported that Mn binds with DNA interacting with the bases and phosphate groups. The present findings are in agree with those of earlier observations. As in $MnSO_4$, Mn^{2+} is present as a divalent cation which plants can absorb easily, and as that cation is known to have certain specific properties like antagonism to iron and the ability to replace Mg^{2+} in enzymatic systems, the action of $MnSO_4$ on plant systems must be involved with action on enzymatic systems. The high solubility of $MnSO_4$ in water and the affinity of Mn^{2+} as cation with chromosomal elements indicated that the observations with *Allium* and *Pisum* are must be due to the fact that with the lower

concentrations certain proportion of the Mn^{2+} taken in by the root tips acted on the cytoplasmic enzymatic systems, leading to spindle disturbance type of abnormalities. With increasing dosage and the accumulation of correspondingly higher amounts of Mn^{2+} to the cells the excess manganese ions left after reacting with the cytoplasmic system upto critical threshold, then acted on the chromosomes and frequency of total aberrations increased.

In comparison to *Allium* sp., *Pisum* showed less number of chromosomal aberrations which can be explained by the fact that due to only 24 hours of soaking prior to recovery the exposure of the chromosomes to the chemicals was for a rather short span of time. The chemical took a certain amount of time in penetrating the seed coat and reaching the targets of individual cell and cell nuclei. Mn^{2+} therefore, had not been able to cross the critical threshold in order to affect the nuclear components.

Amongst mammals, Mn is a known cytotoxic metal on BALB/C mouse 3TC fibroblasts (Borenfreund and Puerner 1986). In Chinese Hamster ovary cells and human diploid fibroblasts Mn^{2+} treatment included true DNA strand breaks (Hamilton-Koch *et al.* 1986, Snyder 1988).

In general, following exposure to $MnSO_4$ there was a progressive increase in frequency of total abnormalities which was directly proportional to the doses. Higher number of breaks per cell is possibly due to the direct action of the available Mn^{2+} ions on the chromosomal material leading to specific breaks and rejoining. Higher concentrations led to greater Mn accumulation within the nucleus and within the cytoplasm of bone marrow cells. Manganese is an intracellular oligo-element which is highly concentrated by the mitochondria and within the nucleus (Maynard and Cotzias 1955, Mena 1980). As a result breaks per cell increases significantly. The total chromosomal abnormalities, however, failed to show a significant trend after 7 days treatment probably due to the more or less similar frequency of pulverisation and spindle disturbance.

Summary

Manganese, a metal of the first group of transitional elements is a known essential element for nutrition in both plant and animal systems. Information on the effects of Mn on cell division and chromosome, particularly *in vivo* is relatively meagre. The present experiment with both plant and animal systems indicated its toxicity. Mn^{2+} induced statistically significant number of breaks in both the systems *in vivo*.

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