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In vivo studies on the effect of *Ocimum sanctum* L. leaf extract in modifying the genotoxicity induced by chromium and mercury in *Allium* root meristems

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Abstract: *In vivo cytogenetic assay in Allium cepa root tip cells has been carried out to detect the modifying effect of Ocimum sanctum aqueous leaf extract against chromium (Cr) and mercury (Hg) induced genotoxicity. It was observed that the roots post-treated with the leaf extract showed highly significant (p<0.001) recovery in mitotic index (MI) and chromosomal aberrations (CA) when compared to pre-treated (Cr/Hg) samples and the lower doses of the leaf extract were found to be more effective than higher doses. The present study reveals that the Ocimum sanctum leaf extract possesses the protective effect against Cr/Hg induced genetic damage.*

Key words : *Ocimum sanctum, Allium cepa, Genotoxicity, Chromium, Mercury.*

Introduction

Disposal of various hazardous chemicals, due to the rapid urbanization and industrialization, environmental pollution has increased markedly over the past decades. Among them heavy metals play a major role in causing several serious problems to the mankind, particularly mutagenicity and carcinogenicity (Nagao, 1978). Compared to organic waste, inorganic waste like heavy metals from various industries, pose a great threat, as they cannot be completely eliminated from the ecosystem. Against this background, a considerable interest has generated to screen the natural substances capable of neutralizing the mutagenic effects. Recent research has confirmed that many naturally occurring substances in plants have protective effects against environmental mutagens and carcinogens (Kada *et al*., 1978; Morita *et al*., 1978; Lai *et al*., 1980; Aruna and Sivaramakrishnan, 1990; Edenharder *et al*., 2003; Salma *et al*., 2004). In the present study *in vivo* assay of chromosomal aberration (CA) and mitotic index (MI) in *Allium cepa* root meristem cells has been carried out to detect the *Ocimum sanctum* aqueous leaf extract in modifying the genotoxicity induced by Cr and Hg which are known mutagen and extensively used heavy metals.

Materials and Methods

Preparation of the extract: Fresh leaves of *Ocimum sanctum* (green variety) were collected and shade dried for two days. 10g of powdered leaves refluxed with 1000 ml of distilled water for 1 hr and filtered. From this 1% extract different dilutions were made i.e. 5, 10, 20, 40, 60, 80, 100%.

Experimental design: The growing roots of *Allium cepa* (2n = 16) were exposed to freshly prepared potassium dichromate (Cr**)** [S.d. fine - chem Ltd, Mumbai] 4 x 10^{-5} M and mercuric chloride (Hg) [E.Merck (India) Ltd, Mumbai] 4.4 x 10^{-5} M for 2 h. Then the roots were washed in running tap water and post-treated with various concentrations of the *O.sanctum* leaf extract and tap water for 4 h. The appropriate positive (Cr and Hg) and negative (tap water) controls were maintained and handled alike. Batches of 5 bulbs per treatment were used. 10 root tips from 5 bulbs of the control and experimental samples were washed in tap water. The root meristems were excised and fixed in acetic acid / ethanol (1:3). Cytological slides were prepared from 10 root tips for each treatment by adopting haematoxylin squash procedure (Marimuthu and Subramaniam, 1960). The coded slides were examined under Nikon microscope to determine the frequency of MI and CA. The differences in the frequencies of MI and CA between control and treated groups were analysed for statistical significance using student's *t* - test.

Results

The results of the effect of *O.sanctum* aqueous leaf extract on MI and CA induced by Cr and Hg in *A.cepa* root tip cells are presented in Table 1 and 2. Cr and Hg treated group showed a drastic reduction in the frequency of mitotic index. This divisional frequency is statistically highly significant when compared to the negative control set ($p<0.001$). The treated roots, post-treated with tap water and leaf extract were showed significant recovery in MI when compared to positive control group ($p<0.05$, $p<0.001$). The occurrence of CA was observed to be high in the roots treated in Cr and Hg when compared to the negative control. This is statistically highly significant ($p<0.001$).

The roots post-treated with leaf extract showed highly significant recovery in CA ($p<0.001$). Though there was reduction in the frequency of CA in the cells post-treated with tap water, it is statistically insignificant. C-metaphase, chromosomal fragments, disturbed anaphase and binucleate cells were the most frequent type of aberration in Cr and Hg treated group. In combination treatment with various doses of leaf extract were found to be effective in reducing the frequency of aberrant cells significantly. Of the different doses of extract

Table – 1: Mitotic indices and chromosomal aberrations observed during pre – treatment (Cr/Hg) and post-treatments with *Ocimum sanctum* aqueous leaf extract and tap water in *Allium* root tip cells.

S.No	Concentration	Total analysed cells	Number of cells showing division	Total aberrant cells	Mitotic index [Mean ± SE]	Chromosomal aberration [Mean ± SE]
1	Control	4250	220	21	5.34 ± 0.36	9.45 ± 1.63
2	Hg	4500	60	46	1.34 ± 0.17**	79.55 ± 6.07**
3	Hg + water	3950	101	66	2.42 ± 0.49*	76.20 ± 5.69
4	Hg + 5% ext	1875	83	27	4.81 ± 0.59**	28.45 ± 8.18**
5	Hg + 10% ext	3250	176	67	5.38 ± 0.46**	38.03 ± 3.05**
6	Hg + 20% ext	3750	215	83	5.77 ± 0.59**	38.55 ± 4.02**
7	Hg + 40% ext	2900	111	46	3.82 ± 0.30**	42.56 ± 3.80**
8	Hg + 60% ext	2000	66	28	3.35 ± 0.17**	39.66 ± 4.61**
9	Hg + 80% ext	3900	211	98	5.52 ± 0.42**	48.25 ± 3.61**
10	Hg + 100% ext	3750	166	97	4.51 ± 0.52**	62.56 ± 4.46*
11	Cr	3925	53	37	1.36 ± 0.31**	72.88 ± 5.48**
12	Cr + water	4600	115	76	2.29 ± 0.33*	64.61 ± 7.22
13	Cr + 5% ext	3350	151	50	4.38 ± 0.33**	27.01 ± 3.84**
14	Cr + 10% ext	3300	126	31	3.89 ± 0.40**	22.06 ± 2.89**
15	Cr + 20% ext	3700	182	45	5.00 ± 0.36**	23.36 ± 3.07**
16	Cr + 40% ext	3350	115	35	3.39 ± 0.41**	27.51 ± 4.57**
17	Cr + 60% ext	3400	112	27	3.31 ± 0.25**	22.55 ± 5.48**
18	Cr + 80% ext	3250	114	35	3.94 ± 0.45**	29.87 ± 3.29**
19	Cr + 100% ext	2650	84	26	3.25 ± 0.37*	31.03 ± 4.58**

* - P<0.05; ** - P<0.001

Table – 2: Types of chromosomal aberrations observed during pre – treatment (Cr/Hg) and post-treatments with *Ocimum sanctum* aqueous leaf extract and tap water in *Allium* root tip cells.

S.No	Concentration	CM %	FG %	LG %	ST %	AB %	DA %	BC %	OT %
1	Control	9.52	--	14.28	14.28	19.04	4.76	--	38.09
2	Hg	13.04	26.08	2.17	26.08	4.34	8.69	13.04	6.52
3	Hg + water	1.51	7.57	3.03	34.84	10.60	15.15	12.12	15.15
4	Hg + 5% ext	7.40	7.40	3.70	51.85	11.11	11.11	--	7.40
5	Hg + 10% ext	--	14.92	5.97	41.79	13.43	--	--	23.88
6	Hg + 20% ext	18.07	7.22	32.53	21.68	2.40	6.02	--	12.04
7	Hg + 40% ext	--	6.52	8.69	65.21	8.69	--	--	10.86
8	Hg + 60% ext	3.57	7.14	3.57	39.28	3.57	17.85	7.14	17.85
9	Hg + 80% ext	2.04	20.48	3.06	40.81	17.34	3.06	7.14	6.12
10	Hg + 100% ext	4.12	9.27	6.18	28.86	17.52	10.30	5.15	18.55
11	Cr	5.40	27.02	8.10	27.02	10.81	10.81	8.10	2.70
12	Cr + water	14.47	19.73	6.57	22.36	14.47	3.94	13.15	5.26
13	Cr + 5% ext	2.00	20.00	18.00	24.00	14.00	--	14.00	8.00
14	Cr + 10% ext	9.67	25.80	19.35	22.58	12.90	6.45	--	3.22
15	Cr + 20% ext	15.55	4.44	6.66	28.88	8.88	13.33	6.66	15.55
16	Cr + 40% ext	17.14	11.42	20.00	5.71	14.28	8.57	--	22.85
17	Cr + 60% ext	22.22	11.11	7.40	7.40	14.81	--	7.40	29.62
18	Cr + 80% ext	11.42	17.14	14.28	20.00	2.85	2.85	2.85	28.57
19	Cr + 100% ext	11.53	11.53	3.84	38.46	3.84	11.53	3.84	15.38

CM – C-Metaphase; FG – Fragments; LG – Laggards; ST – Stickiness; AB – Anaphasic bridges; DA – Disturbed anaphase; BC – Binucleate cells; OT – Others.

tested, the lower doses (5, 10, 20%) were found to be more effective in reducing the mitotoxicity and clastogenicity of Cr and Hg than higher doses.

Discussion

Heavy metals are non-degradable and are not acted upon by microbes. They not only accumulate, but are often biologically magnified with their subsequent movement in food chains and biochemical cycle. Cr and Hg, the important heavy metals are widely used in various industries and their mutagenic and carcinogenic effects have been studied in both plant and animal cells (Venett and Levy, 1974; Majone, 1977; Tsuda and Kato, 1979; Flessel *et al*., 1980; Sharma *et al*., 1988; Kumar and Tripathi, 2003).

Ocimum sanctum Linn. a well known medicinal plant has versatile role in traditional medicine. Several researches are being conducted regarding the efficacy of whole plant or its parts for treatment of various diseases (Gupta *et al.*, 2002). Recent studies have proved that *O.sanctum* possess anti-mutagenic, anti-tumourogenic (Annapurani and Priya, 1999), anti-carcinogenic, and radio-protective effects (Umadevi and Ganasoundari, 1995; Umadevi, 2001). It is evident from the results of present study that the aqueous leaf extract of *O.sanctum* has inhibitory effect on the genetic damage induced by Cr and Hg. However, it is not possible from the present study to suggest any plausible mechanism of action, since the extract used in the study contain number of compounds like eugenol, methyl eugenol, sesqueterpenes, flavonoids and phenolic compounds etc. and these compounds individually or synergistically may lead to significant anti-clastogenic effects as observed in the present study. Hence it is worthwhile to look for specific compound(s) in the extract with protective action.

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