CYTOGENETIC TOXICITY OF LEAF EXTRACT OF *PUTRANJIVA ROXBURGHII*, A MEDICINAL PLANT

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ABSTRACT — Young weaning Swiss albino mice were orally administered leaf extract of *Putranjiva roxburghii* at 0.5, 1.0 or 2.0 g/kg body weight/ day for seven consecutive days. The results showed that the leaf extract significantly induced mitosis-disruptive chromosomal changes in bone marrow cells. No change in the incidence of structural abnormalities was, however, noticed among the metaphase chromosomes. It is proposed that the extract might have interfered with the spindle and other proteins causing polyploidy, aneuploidy, c-mitosis, etc.

KEY WORDS: *Putranjiva roxburghii*, Isothiocyanate, Genotoxicity, Mitotic-poison

INTRODUCTION

The use of plants and products thereof for curing and managing various ailments has been known to the world since time immemorial, and is gaining momentum these days due to toxic effects by synthetic drugs. Crude extracts of local plants are frequently being used by traditional communities like tribal and rural populations of India and elsewhere. This, and medicines manufactured on principles of natural compounds even by pharmaceutical companies, may lead to a large-scale exposure of humans to natural products. Though apparently believed non-toxic, a clean chit offered to these plant products requires strict scientific tests, besides clinical ones on different vital systems, more so because these natural products may contain a few harmful ingredients in them as secondary metabolites (Nakamura and Yamamoto, 1982) which may have perilous side effects including mutagenic potentials. It is, therefore, desirable to evaluate the cytogenetic toxicity, if any, of materials of plant origin. The aim of the present work is precisely to assess the genotoxic potential of the crude extract from the leaves of *Putranjiva roxburghii* (Euphorbiaceae) in a suitable laboratory mammal. Since the extract is taken by ignorant subjects a curative dose is seldom in practice. Even Ayurvedic, Unani and Siddha texts (Materia Medica of Plants) are

silent on it. As such, the maximum tolerated dose (Bateman, 1984), which does not produce any significant change in the general physical profile of the mice, was considered as a dose suitable for *in vivo* toxicity test (Kirkland, 1993). Curative effect of the leaf extract of this plant is reported in bile disorders, burns, cancer, cold, dehydration, fever, inflamed throats, and also for aphrodisiac (Krishnamurthy, 1969) and pregnancy terminator properties (Hemadri and Rao, 1983).

MATERIALS AND METHODS

Extract preparation

Mature leaves from the *Putranjiva* tree (about 10 yrs old) were collected from Bhagalpur (Bihar, India) during the flowering season (in the month of March or spring) and were washed thoroughly in running tap water. Air-dried (in shade for about 5 hr) leaves of 250 g weight were kept in an oven for about 36 hr at 60°C and weighed again, at about 245 g, before being coarsely powdered in a glass mortar with the aid of a pestle. 200 g of the powdered leaves were then Soxhlet-extracted with 80% ethanol (v/v) at 60°C using a Soxhlet apparatus (Borocil, Bombay, India) for about 98 hr for full extraction, i.e., till the return of the usual shine of the solvent ethanol. The extract was concentrated *in vacuo* under reduced pressure using a rotatory evapora-

tor (Purfit, AV2, Ambala, India) to retrieve ethanol from the extract solution (which takes a minute or two). The extract was finally dried on a water-bath (at 60°C) to obtain it in the powdered form, which was found to be about 12 g in weight. The green-colored extract thus obtained was kept in an air-tight desiccator (in sample tubes) at room temperature (25 ± 2°C).

Experimental animal

Four - 6 week-old laboratory-bred Swiss albino mice *Mus musculus* (S-cdri, 2n=40, seed colony obtained from Central Drug Research Institute, Lucknow, India) of body weight (bw) 22 ± 3 g, matched for their sex, were used. There was a total of five groups of experimental animals, each comprising six mice (three males and three females). All the experimental animals were housed in stainless steel cages in a room maintained at 25 ± 2°C with 12 hr day/ night cycle. "Gulmuhar" (Hindustan Lever, Bombay, India) diet was the basal food for all the experimental animals. Drinking water was made available *ad libitum*.

Treatment protocol

1. Treated group

Each time freshly prepared water soluble extract was administered to the animals (treated group) at the rate of (@) 0.5 g (T₁), 1.0 g (T₂) or 2.0 g (T₃)/ kg body weight (bw)/ day by dissolving the extract in 0.4 ml of glass double distilled (gdd) water. These doses are in fact equivalent to quarter, half and full maximum tolerated doses, respectively (Bateman, 1984). The treatment was given once daily through oral gavage (by intubation) for seven consecutive days, and the duration from harvest of leaves to the treatment of the extract was about 15 days.

Control group

1. Solvent control

The mice of the group were given 0.4 ml gdd water per day, which is the amount equal to that used for dissolving the extract.

2. Positive control

The mice of this positive control group were dosed with aflatoxin-infested bread (Sinha and Dharmshila, 1994) that had 50 ± 10 ppb of AFB₁, a well-established biomutagen of natural origin, elaborated as a secondary metabolite by the toxigenic strain (BG-185) of *Aspergillus flavus* (ascomycetes fungus). The amount of aflatoxin B₁ thus administered was 0.05 µg/kg bw/day.

Experimental protocol

The experimental animals were injected colchicine (Sisco Laboratory, India) intraperitoneally (i.p.) @ 4 mg / kg bw, 24 hr after the last treatment and 90 min prior to sacrifice by cervical dislocation. Bone marrow from both the dissected-out femora was flushed in hypotonic KCI (0.075 M) solution and the slides were prepared by standard, fixative (1:3 aceto-methanol)-flame drying-Giemsa (1.20) staining technique (Preston *et al.*, 1987). About 600 well-spread metaphase plates (@ about 100 plates / animal) from each group were screened randomly from double-coded slides for detecting chromosome-related abnormalities, if any. The abnormalities detected were put either into two categories, viz., structural changes consisting of chromatid breaks (ct) and gaps (cg) - the later being lesion(s) in one or both the helices of DNA which failed to reconstitute and appeared as unstained attenuated regions having a diameter less than one chromatid, acentric fragment (af), centric fusion (cf) and stickiness (st) and mitosis-disruptive changes such as aneuploidy (a gain, hyperploidy or loss, hypoploidy of one or more chromosomes), polyploidy, c-mitosis and precocious separation.

Statistical analysis

The data from all the animals in each group were pooled together and the Equality of Proportion Test (z-test), using $p < 0.05$ as the minimum acceptable criterion (level) of significance, was performed for statistical evaluation (Downie and Health, 1970).

RESULTS

The total abnormality frequency (T₁ : 7.8%, T₂ : 9.3%, T₃ : 10.2%) among the leaf-extract-fed groups was significantly higher than that of the solvent control (5.1%) at all doses (Table 1), but significantly less in comparison to that of the positive control (14.2%). The treatment with the leaf extract however, failed to induce any significant increase in the frequency of structural changes (T₁ : 3.3%, T₂ : 3.9%, T₃ : 3.7%) and remained almost equal to that of the solvent control (3.3%). These frequencies were found to be significantly low (T₁ < .001, T₂ and T₃ < .05) when compared with that of the positive control (7.6%). But, the frequency of division-disruptive changes in treated groups (T₁ : 4.5%, T₂ : 5.4%, T₃ : 6.4%) increased significantly with respect to the solvent control (1.8%), though not different from that of the positive control group (6.5%). The effect dose not appear to be sex-influenced.

DISCUSSION

The results thus show that the leaf extract of *P. roxburghii* is not clastogenic, because it does not produce structural changes in chromosomes of bone marrow cells of mice. The significant increase in the incidence of numerical variations in chromosomes indicates that the extract is acting as a mitotic poison. It is

the nature of mitotic poisons that they do not necessarily react with DNA (Au and Hsu, 1982), but interfere with the functioning or assemblage of the spindle apparatus (Awasthy *et al.*, 1995 and 1999). The exact action mechanism of even the best-known mitotic poison, colchicine, is yet to be elucidated. However, it is proposed that they produce microtubule specific metabolites and active radicals upon being biotransformed in

Table 1. Frequency (% $\pm$ S.E.) of chromosomal abnormalities in bone marrow cells of mice belonging to various experimental groups (N = $\sim$ 600 metaphase plates screened @ about 100 plates / animal).

Experimental groups (dose/kg bw/day)		Sex (no. of metaphases)	Structural types							Mitosis-disruptive types						Total no. %±S.E.
			ct	cg	dl	st	af	cf	Total no. %±S.E.	an		po	cm	pc	Total no. %±S.E.	
										ho	hy					
C O N T R O L	Solvent (20 ml gdd)	M (305)	2	4		3	2		11 (3.6±1.1)	2		1	1		4 (1.3±0.7)	15 4.9±1.2
		F (302)	4		1	1	3		9 (2.9±0.9)	3		2		2	7 (2.3±0.9)	16 5.3±1.3
		Total (607)	6	4	1	4	5		20 (3.3±0.7)	5		3	1	2	11 (1.8±0.5)	31 5.1±0.9
	Positive (0.05 μg AFB1)	M (315)	8	3	4	4	4		23 (7.3±1.5) ^a	8		4	5	3	20 6.4±1.4) ^c	43 13.7±1.9 ^c
		F (300)	10	2	1	2	5	4	24 (8.0±1.6) ^b	4	2	7	5	2	20 (6.7±1.4) ^b	44 14.6±2.1 ^c
		Total (615)	18	5	5	6	9	4	47 (7.6±1.1) ^c	12	2	11	10	5	40 (6.5±0.9) ^c	87 14.2±1.4 ^c
T R E A T E D	T1 (0.5 g extract)	M (330)	5		3		1	2	11 (3.3±0.9) ^d	4		5		4	13 (3.9±1.1) ^a	24 7.3±1.4 ^c
		F (311)	4	2	2			2	10 (3.2±0.9) ^e	6	2	3	2	3	16 (5.2±1.3)	26 8.4±1.5 ^d
		Total (641)	9	2	5		1	4	21 (3.3±0.7) ^f	10	2	8	2	7	29 (4.5±0.8) ^b	50 7.8±1.1 ^{af}
	T2 (1.0 g extract)	M (310)	6	1	1			2	10 (3.2±0.9) ^d	5	1	3	5	4	18 (5.8±1.3) ^c	28 9.0±1.6 ^a
		F (322)	7			3	2	3	15 (4.7±1.2)	8	1	1	4	2	16 (4.9±1.2)	31 9.6±1.6 ^{ad}
		Total (632)	13	1	1	3	2	5	25 (3.9±0.8) ^e	13	2	4	9	6	34 (5.4±0.9) ^c	59 9.3±1.2 ^{be}
	T3 (2.0 g extract)	M (319)	5	2		1		2	10 (3.1±0.9) ^d	9	3	5	3	2	22 (6.9±1.4) ^c	32 10.0±1.7 ^a
		F (302)	5	2	1		1	4	13 (4.3±1.2)	8		2	1	7	18 (5.9±1.4) ^a	31 10.3±1.8 ^a
		Total (621)	10	4	1	1	1	6	23 (3.7±0.8) ^e	17	3	7	4	9	40 (6.4±0.9) ^c	63 10.2±1.2 ^{ce}

Abbreviations: ct = chromatid break, cg = chromatid gap, dl = deletion, af = acentric fragment, an = aneuploidy, ho = hypoploidy, hy = hyperploidy, po = polyploidy, cf = centric fusion, cm = c-mitosis, pc = precocious separation, st = stickiness. Symbols: a or d, b or e and c or f indicate a significant difference at 0.05, 0.01 and 0.001 levels, respectively, over solvent control or positive control.

the cell (Sies, 1993). These metabolites and radicals may directly bind to tubulin dimers at the assembly end or bind to a free sub-unit, to be incorporated, thereby blocking further addition of monomers. Meanwhile they may initiate depolymerization at the opposite end of the microtubules, leading to their dissolution (Karp, 1996). Thus the extract seems to have inhibited the synthesis and / or induced the destruction of the proteins responsible for spindle formation leading to unequal distribution of chromosomes and causing mitosis-disruptive changes as noted in the present study.

The extract from leaves and shoots contains isopropyl- and 2-butyl-isothiocyanates as its main and 2-methyl-butyl-isothiocyanates as its minor component; some amounts, of mannitol, saponin, glucosides and other unidentified alkaloids are also detected (Krishnamurthy, 1969). Chemopreventive agents such as benzyl isothiocyanate, phenethyl isothiocyanate, morbonyl isothiocyanate etc. have been found to act as antioxidants or antimutagenic agents (Kusamran *et al.*, 1998). The major and minor isothiocyanates, much like most of their isoforms, have might been involved in mitigating the mutagenic effects produced by other constituents of the extract or they might have produced genotoxicities of their own in a manner similar to some natural antioxidants (Stich, 1991). These mechanisms currently proposed, though based on related facts or beliefs are, however, mere speculations, and therefore, demand studies with isolated principle factors in the future. Since, the extract does produce mitotic impairments in dividing bone marrow cells of mice, the action is mutagenic (toxic), and therefore cannot be recommended for medicinal purposes at the doses presently tested.

REFERENCES

- Au, W. and Hsu, T.C. (1982): Assays for chromosomes aberrations using mammalian cells in culture. In Cytogenetic Assays of Environmental Mutagens (Hsu, T.C., ed.), pp. 203-213, Oxford and IBH Publishing Co. New Delhi.
- Awasthy, K.S., Chaurasia, O.P. and Sinha, S.P. (1995): Genotoxic effects of crude extract of neem (*Azadirachta indica*) in bone marrow cells of mice. Cytologia., **60**, 189-193.
- Awasthy, K.S., Chaurasia, O.P. and Sinha, S.P. (1999): Prolonged murine genotoxic effects of crude extracted from neem. Phytotherapy Res., **13**, 81-83.
- Bateman, A.J. (1984): The dominant lethal assay in the male mouse. In Handbook of Mutagenic Test Procedures. (Kilbey, B.J., Legator, M., Nichols, M. and Ramel, C., ed.), 2nd ed., pp. 471-484. Elsevier, Amsterdam.
- Downie, N.M. and Health, R.W. (1970): Basic Statistical Methods. 3rd ed. pp. 86-239. A Harper International Edition, Harper and Row Publishers. Inc. New York.
- Hemadri, K. and Rao, S.S. (1983): Antifertility, abortifacient and fertility promoting drugs from Dandakaranya. Ancient Science of Life, III, 255-264.
- Karp, G. (1996): Cytoskeleton and Cell motility (Microtubule Organizing Centers). In Cell and Molecular Biology. Concept and Experiments (Rubin, K., ed.), pp. 350, John Wiley & Sons, Inc., Von Hoffmann press, U.S.
- Kirkland, D.J. (1993): Genetic toxicity testing requirements: Official and unofficial views from Europe. Mol. Toxicol., **21**, 8-14.
- Krishnamurthy, A. (1969): *Putranjiva roxburghii*. In Wealth of India, Raw materials (Krishnamurthy, A., ed.), revised ed., Vol. 8, pp. 325, Publication and Information Directorate. CSIR, Indian Printing Works, New Delhi.
- Kusamran, W.R., Ratanavila, A. and Tepsuwan, A. (1998): Effect of neem flowers, Thai and Chinese bitter gourd fruits and sweet basil leaves on hepatic monooxygenases and glutathione s-transferase activities and *in-vitro* metabolic activation of chemical carcinogens in rats. Food Chem. Toxicol., **36**, 475-484.
- Nakamura, H. and Yamamoto, T. (1982): Mutagen and antimutagen in ginger, *Zingiber officinale*. Mut. Res., **103**, 119-126.
- Preston, R.J., Dean, B.J., Galloway, S., Holdon, H., Mc Fee, A.F. and Shelby, M. (1987): Mammalian *in-vivo* cytogenetic assay. Analysis of chromosome aberrations in bone marrow cells. Mut. Res., **189**, 157-165.
- Sies, H. (1993): Strategies of antioxidant defence. Eur. J. Biochem., **215**, 213-219.
- Sinha, S.P. and Dharmshila, K. (1994): Vitamin A ameliorates the genotoxicity in mice of aflatoxin B₁-containing *Aspergillus flavus* infested food. Cytobios, **79**, 85-95.
- Stich, H.F. (1991): The beneficial and hazardous effects of simple phenolic compounds. Mutat. Res., **259**, 307-324.