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Treatment of mice with a herbal preparation (Liv.52) reduces the frequency of radiation-induced chromosomal damage in bone marrow

Ganesh Chandra Jagetia and **Ganapathi, N.G.,**
Department of Radiobiology, Kasturba Medical College, Manipal, Karnataka, India.

ABSTRACT

Induction of chromosomal aberrations was studied from ¼ to 14 days post irradiation in the bone marrow of mice treated or not treated with Liv.52, a herbal preparation, prior to 4.5 Gy exposure. The frequency of chromatid and chromosomal aberrations started increasing at day ¼ in the irradiated and Liv.52 + irradiated groups. The highest frequency of aberrations was recorded at day ½ post exposure, which declined after day 1 in both groups. The frequency of both types of aberrations was significantly lower in the Liv.52 + irradiated group than in the irradiated group.

INTRODUCTION

Liv.52 is a non-toxic herbal preparation composed of *Capparis spinosa, Cichorium intybus, Solanum nigrum, Cassia occidentalis, Terminalia arjuna, Achillea millefolium* and *Tamarix gallica*. It has been reported to be clinically effective in treating hepatotoxicity and a wide range of hepatic disorders (Mathur, 1975; Sule *et al.,* 1968; Deshpande *et al.* 1971). The radioprotective potential of Liv.52 was demonstrated for the first time in mice by Saini *et al.* (1984a, b) against radiation-induced sickness, dermatitis and spleen injury. Recently, Jagetia and Ganapathi (1989) have reported that prior administration of Liv.52 reduced the formation of micronuclei in mouse bone marrow.

The present study was undertaken to elucidate the protective action of Liv.52 against radiation-induced chromosomal aberrations in bone marrow of mice exposed to 4.5 Gy of whole-body γ–radiation.

MATERIAL AND METHODS

Male Swiss albino mice, 6-8 weeks old and weighing 26.9 ± 2.54 g, were selected from an inbred colony maintained under controlled conditions of temperature (23 ± 2°C), humidity (50 ± 5%) and light (10 and 14 h of light and dark). The animals were given sterile food (wheat 70%, Bengal gram 20%, fishmeal 5%, yeast powder 4%, sesame oil 0.75% and shark liver oil 0.25%) prepared in the laboratory and water *ad libitum*. Throughout the experiment 5-6 animals were housed in a polypropylene cage containing sterile paddy husk (procured locally) as bedding.

One group of animals was fed orally (using 22 gauge oral feeding needles) with a 5% dextrose solution once a day for 7 days before irradiation and served as the control group, while the other mice received 500 mg/kg b.wt. of Liv.52 powder (supplied by The Himalaya Drug Company) in 5% dextrose solution in a similar fashion. One hour after administration on day 7, the animals of both groups were exposed to 4.5Gy of γ-radiation (Gammatron telecobalt therapy source) in specially designed well-ventilated plastic boxes. The animals were irradiated in groups of 10 at a dose rate 0.88 Gy/min., at a distance of 60 cm from the source (dosimetry done by Dr. J.C.R. Solomon,

Department of Radiotherapy and Oncology, K.M.C. Manipal). For comparison a few animals were also treated with 5% dextrose and Liv.52 as above but without irradiation.

Animals from each group were given 0.025% colchicine intraperitoneally 2 h before killing. The animals from each group were killed by cervical dislocation at ¼, ½, 1, 2, 3, 7 and 14 days post exposure. The femora were removed and the metaphase plates were prepared by the usual cytogenetic method. The slides were stained in 4% Giemsa at pH 6.8 The chromosomal aberrations (chromatic and chromosomes) were scored on an AO Reichert Microse at a magnification of 500 x. Four hundred metaphase plates were scored from each animal and a total of 2000 metaphase plates were scored for 5 animals at each post-irradiation time. The criteria for scoring were based on the classification of Savage (1975).

The data were analysed by the chi square test on an IBM computer.

RESULTS AND DISCUSSION

The results are expressed as aberrations/100 cells. Liv.52 administration did not affect the control frequency of aberrations (Table 1).

Table 1: Frequency of chromosomal aberrations in the bone marrow of mice exposed to 4.5 Gy^{60} Co g rays with or without Liv.52 treatment

Post-irradiation time (days)	Treatment	Aberrant cells	Aberrations per 100 cells						Total aberrations	Polyploids (%)	Pulverization (%)
			Chromatid breaks	Chromosome breaks	Centric rings	Dicentrics	Exchanges	Acentric fragments			
Sham-irradiation		0.50 ± 0.07	0.10	0.00	0.10	0.00	0.00	0.50	0.70	0.15	0.00
Liv.52+Sham-irradiation		0.55 ± 0.09	0.15	0.00	0.00	0.00	0.00	0.60	0.80	0.10	0.00
		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
¼	C	9.15 ± 0.58	0.90	0.00	0.00	0.00	0.00	12.35	13.25	0.00	0.70
	E	7.25 ± 0.37	0.50	0.00	0.00	0.00	0.00	7.45	7.95	0.00	0.45
		p <0.05	NS	NS	NS	NS	NS	p <0.001	p <0.001	NS	NS
½	C	51.45 ± 6.00	11.05	4.05	2.65	4.20	1.30	123.25	146.50	0.55	2.65
	E	26.45 ± 3.78	3.50	1.70	2.15	1.60	0.50	62.85	72.30	0.50	0.25
		p <0.001	p <0.001	p <0.001	NS	p <0.001	p <0.01	p <0.001	p <0.001	NS	p <0.001
1	C	45.25 ± 3.32	3.50	5.00	5.35	0.60	1.15	81.80	97.40	1.95	3.35
	E	24.40 ± 1.07	3.25	3.00	3.60	0.50	0.30	53.40	63.90	1.65	2.15
		p <0.01	NS	p<0.01	p<0.01	NS	p <0.01	p <0.001	p <0.001	NS	p <0.05
2	C	11.25 ± 0.97	0.65	0.45	0.35	0.25	0.35	13.40	15.45	0.85	0.00
	E	5.80 ± 0.76	0.20	0.35	0.20	0.05	0.05	7.50	8.35	0.60	0.00
		p <0.001	p <0.05	NS	NS	NS	p <0.05	p <0.001	p <0.001	NS	NS
3	C	7.10 ± 1.16	0.70	0.05	0.20	0.15	0.25	10.50	11.85	0.25	0.00
	E	2.70 ± 0.28	0.10	0.05	0.05	0.05	0.05	3.80	4.10	0.20	0.00
		p <0.001	p <0.01	NS	NS	NS	NS	p <0.001	p <0.001	NS	NS
7	C	3.05 ± 0.28	0.35	0.10	0.00	0.00	0.00	4.75	5.20	0.00	0.00
	E	2.10 ± 0.30	0.10	0.00	0.00	0.00	0.00	3.70	3.80	0.00	0.00
		p <0.05	NS	NS	NS	NS	NS	NS	p <0.05	NS	NS
14	C	2.80 ± 0.09	0.15	0.15	0.00	0.00	0.10	3.65	4.05	0.00	0.00
	E	1.80 ± 0.25	0.00	0.00	0.00	0.00	0.00	3.35	3.35	0.00	0.00
		p <0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS

C – irradiated group; E – Liv.52 + irradiated group.
400 metaphases were scored/animal and 5 animals were used for each group at each post exposure time.

The frequency of aberrant cells increased from day ¼ reaching to a peak level on day ½ in both the irradiated and Liv.52 + irradiated groups and declined thereafter. The frequency of aberrant cells was significantly less in the Liv.52 + irradiated group than in the irradiated group (Table 1).

Chromatid breaks were seen at day ¼, which continued to increase up to day ½ and decline thereafter. The normal levels were restored on days 14 and 2 in the irradiated and Liv.52 + irradiated group respectively (Table 1).

Chromosome breaks, centric rings, dicentrics and chromosome exchanges were observed at day ½ post exposure in both groups. The frequency of chromosome breaks and centric rings was highest on day 1, while that of dicentrics and exchanges was highest on day ½ and declined thereafter. The aberration frequency was always lower in the Liv.52 + irradiated group than in the irradiated group (Table 1).

The highest frequency of total aberrations was recorded on day ½ in both groups, thereafter it declined continuously up to day 14 post irradiation. The percentage of total aberrations was significantly lower in the Liv.52 + irradiated group up to day 7 than in the irradiated group (Table 1).

Pulverized cells increased from day ¼ and the highest number of these cells was recorded on day 1; thereafter pulverized cells could not be observed. The frequency of pulverized cells was significantly lower in the Liv.52 + irradiated group than in the irradiated group (Table 1).

DISCUSSION

Liv.52 has been reported to protect mice against radiation-induced sickness, dermatitis and spleen injury (Saini *et al,* 1984a,b). The administration of Liv.52 prior to irradiation resulted in a significant decline in chromosomal aberrations (Table 1). The frequency of aberrant cells was significantly lower in the Liv.52 + irradiated group than in the irradiated group at all post-exposure time periods studied. Gupta and Uma Devi (1985, 1986) and Thomas and Uma Devi (1987) have also reported a decline in aberrant cells in mice treated with MPG and WR-2721, singly or in combination, before exposure to γ radiation.

Liv.52 was equally effective in protecting the chromosomes against radiation-induced damage, as is evidenced by the lower frequency of chromatic breaks, chromosome breaks, centric rings, dicentrics, exchanges, acentric fragments and total aberrations. Most of these aberrations had returned to the normal level at day 3 post exposure in the Liv.52 + irradiated group as compared to the irradiated group. These findings support our earlier report on micronuclei, where the frequency of micronuclei in the Liv.52 + irradiated mice returned to a normal level at day 3 post exposure (Jagetia and Ganapathi, 1989).

The frequency of pulverized cells was always significantly lower in the drug-treated group than in the irradiated group. This indicates that Liv.52 could protect the cells against the severe infliction of radiation damage.

The exact mechanism by which Liv.52 prevents chromosomal damage is not known. The depletion of intracellular glutathione (GSH) has been reported to be one of the causes of radiation-induced damage, while increased levels of intracellular GSH are responsible for radioprotective action. A similar mechanism of action may be attributed to the radioprotective action of Liv.52, which has been reported to restore the intracellular GSH level to normal in rats exposed to 4.0 Gy of γ radiation (Sarkar *et al.*, 1989).

There is growing evidence that double-strand breaks (dsb) are mainly responsible for the formation of chromosomal aberrations (Natrajan *et al.,* 1980; Bryant, 1988). It is possible that the elevated levels of GSH in the Liv.52-treated group may be able to enhance the repair of dsb, lowering the frequency of chromosomal aberrations in this group. This conclusion is supported by the recent study of Ochi (1989) who has reported that chromosomal aberrations were repaired only in GSH-positive cells.

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