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Mentha piperita (Linn.) leaf extract provides protection against radiation induced chromosomal damage in bone marrow of mice

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Oral administration of *M. piperita* (1 g/kg body weight/day) before exposure to gamma radiation was found to be effective in protecting against the chromosomal damage in bone marrow of Swiss albino mice. Animals exposed to 8 Gy gamma radiation showed chromosomal aberrations in the form of chromatid breaks, chromosome breaks, centric rings, dicentrics, exchanges and acentric fragments. There was a significant increase in the frequency of aberrant cells at 6 hr after irradiation. Maximum aberrant cells were observed at 12 hr post-irradiation autopsy time. Further, the frequency of aberrant cells showed decline at late post-irradiation autopsy time. However, in the animals pretreated with *Mentha* extract, there was a significant decrease in the frequency of aberrant cells as compared to the irradiated control. Also significant increase in percentage of chromatid breaks, chromosome breaks, centric rings, dicentrics, exchanges, acentric fragments, total aberrations and aberrations/damaged cell was observed at 12 hr post-irradiation autopsy time in control animals, whereas *Mentha* pretreated irradiated animals showed a significant decrease in percentage of such aberrations. A significant decrease in GSH content and increase in LPO level was observed in control animals, whereas *Mentha* pretreated irradiated animals exhibited a significant increase in GSH content and decrease in LPO level but the values remained below the normal. The radioprotective effect of *Mentha* was also demonstrated by determining the $LD_{50/30}$ values (DRF=1.78). The results from the present study suggest that *Mentha* pretreatment provides protection against radiation induced chromosomal damage in bone marrow of Swiss albino mice.

Mentha piperita (Linn.) commonly called as peppermint belonging to the family Labiatae is considered aromatic, stimulant and carminative. It is being used for allaying nausea, flatulence and vomiting[1]. *Mentha* extract has been shown to have antioxidant and antiperoxidant properties due to the presence of eugenol, caffeic acid, rosmarinic acid and α-tocopherol[2-4]. *Mentha* extract and its oil have also been screened for antibacterial and antifungal activities against *Pseudomonas solanacearum* and *Aspergillus niger*, *Alternaria alternata* and *Fusarium chlamydosporum*, respectively[5,6]. Vokovic-Gacis and Simic[7] showed that extracts of mint (*Mentha*) could enhance error-free repair of damage and, hence, could be antimutagenic. Samman *et al.*[8] reported that *Mentha piperita* has a chemopreventive effect against shamma (a complex mixture of powdered tobacco, slaked lime, oils, spices and other additives, has been linked to oral cancer in Saudi Arabia) induced carcinogenesis, which could be due to antimutagenic properties. It has been reported that *Mentha* treatment protects the hematological constituents and serum phosphatases activity in Swiss albino mice against gamma irradiation[9,10]. Recently, Samarth *et al*,[11] showed that *Mentha piperita* (Linn.) leaf extract provides protection against radiation induced alterations in intestinal mucosa of Swiss albino mice. Therefore, the present study has been undertaken to evaluate the radioprotective effect of leaf extract of *Mentha piperita* Linn. in terms of chromosomal aberrations in bone marrow of Swiss albino mice.

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Materials and Methods

Animals — Adult male Swiss albino mice (6-8 weeks old, weighing 25 ± 2 g) maintained in the animal house as an inbred colony (procured from Hamdard University, Delhi) were used. These were maintained on standard mice feed and water *ad libitum*.

Irradiation — The Cobalt teletherapy unit (ATC-C9) at cancer treatment centre, Radiotherapy Department, SMS Medical College and Hospital, Jaipur was used for irradiation. Unanaesthetised mice restrained in well-ventilated Perspex boxes were exposed to whole-body gamma radiation (8 Gy) at a distance (SSD) of 77.5 cm from the source to deliver the dose-rate of 1.59 Gy/min. The dose was calculated as per the physical decay table for Co^{60}.

Mentha extract (ME)—Fresh leaves of *Mentha piperita* Linn. [RUBL-19443], collected locally were air dried, powdered and extracted with double distilled water (DDW) by refluxing for 36 hr (12 hr×3) at 80°C. The extract thus obtained was vacuum evaporated so as to make it in powder form. The extract was redissolved in DDW just before oral administration.

Experimental design—Mice selected from inbred colony were divided into two groups. Animals of one group were administered ME orally (1 g/kg body weight/day) for three consecutive days to serve as experimental, while the other group received DDW (volume equal to ME) to serve as control. On 3rd day, after 30 min of above treatments animals of both the groups were exposed to 8 Gy gamma radiation.

Survival assay—Mice, both control and experimental, exposed whole-body to gamma radiation (4, 6, 8 and 10 Gy) were checked daily for 30 days. The survival percentage of mice up to 30 days of exposure against each radiation dose was used to construct survival-dose-response curves.

Cytogenetic study—Cytogenetic damage in the bone marrow cells was studied by chromosomal aberration analysis. All the animals were injected (i.p.) with 0.025% colchicine and sacrificed 2 hr later by cervical dislocation. Both femurs were dissected out. Metaphase plates were prepared by the air drying method[12]. Bone marrow from the femur was aspirated, washed in saline, treated hypotonically (0.6% sodium citrate), fixed in 3:1 :: methanol:acetic acid, dried and stained with 4% Giemsa (Sigma, USA). Chromosomal aberrations were scored under a light microscope. A total of 400 metaphase plates were scored per animal. Different types of aberration like chromatid breaks, chromosome breaks, fragments, rings, exchanges and dicentrics were scored. When breaks involved both the chromatids, it was termed "chromosome type" aberration, while "chromatid type" aberration involved only one chromatid. If the deleted portion had no apparent relation to a specific chromosome, it was called a fragment[13].

Biochemical study—Reduced glutathione (GSH) assay: The hepatic level of reduced glutathione (GSH) was determined by the method as described by Moron *et al*[14]. GSH content in blood was measured Spectrophotometrically using Ellman's reagent (DTNB) as a colouring reagent as per the method described by Beutler *et al*[15]. The absorbance was read at 412 nm using a UV-VIS Systronics Spectrophotometer.

Lipid peroxidation assay: The lipid peroxidation level in liver and serum was measured using Thiobarbituric Acid Reactive Substances (TBARS) by the method of Ohkhawa *et al*[16]. The absorbance was read at 532 nm.

Statistical analysis—The data were subjected to Student's t test for comparison between the groups. The values are expressed as mean ± SE. Significance level was set at $P<0.05$, <0.005 and <0.001.

Results

In the present investigation, it was observed that pre-treatment of *Mentha* enhanced the survival percentage of mice exposed to different doses of gamma radiation. *Mentha* pre-treatment inhibited mortality completely at 4 and 6 Gy. However, at 8 and 10 Gy, no animal died before day 7, and only 18 and 42% death occurred between day 7 and 10 (Fig. 1). Radiation dose-response curves for mice with or without pretreatment of *Mentha* are shown in Fig. 2. The $LD_{50/30}$ for control (irradiation alone) and experimental (*Mentha* extract+irradiation) animals were computed as 6.48 ± 0.07 and 11.59 ± 0.21 Gy, respectively. On the basis of $LD_{50/30}$ values, *Mentha* pretreatment produced a dose reduction factor (DRF) of 1.78.

The present study also reports the radioprotective activity of *M. piperita* leaf extract in terms of chromosomal aberrations in bone marrow of Swiss albino mice. Animals exposed to 8 Gy gamma radiation showed chromosomal aberrations in the form of chromatid breaks, chromosome breaks, centric rings, dicentrics, exchanges and acentric fragments (Fig. 3). There was a significant increase in the frequency of aberrant cells at 6 hr after irradiation. Maximum aberrant cells were observed at 12 hr post-irradiation. Further, the frequency of aberrant cells showed decline at late post-irradiation autopsy time. However, in the animals pretreated with *Mentha* extract, there was a significant decrease in the frequency of aberrant cells as compared to the irradiated control (Fig. 4). Also significant increase in percentage of chromatid breaks, chromosome breaks, centric rings, dicentrics, exchanges, acentric fragments, total aberrations and aberrations/damaged cell was observed at 12 hr post-irradiation autopsy time in control animals, whereas *Mentha* pretreated irradiated animals showed a significant decrease in percentage of such aberrations (Table 1; Figs 5,6).

No significant variation in the hepatic as well as blood GSH content was observed between normal and

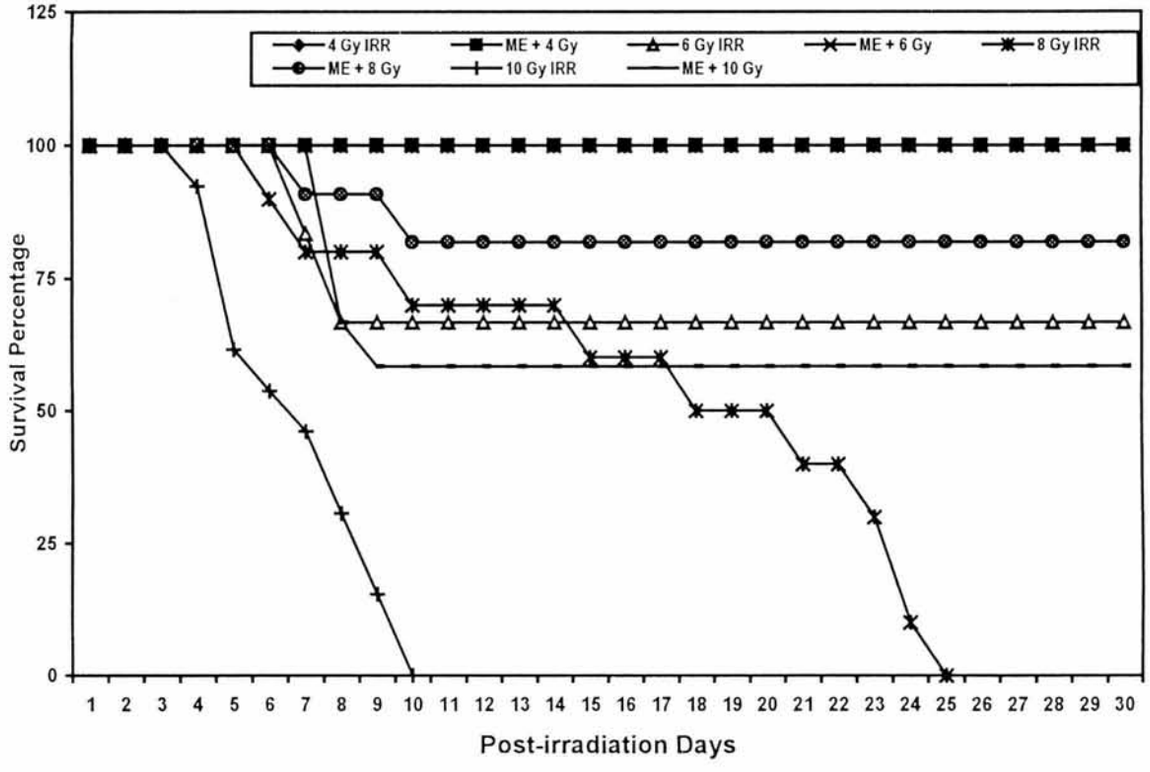

Fig. 1 — Survival of mice (30 days) with or without *Mentha* extract (ME) treatment after exposure to different doses of gamma radiation

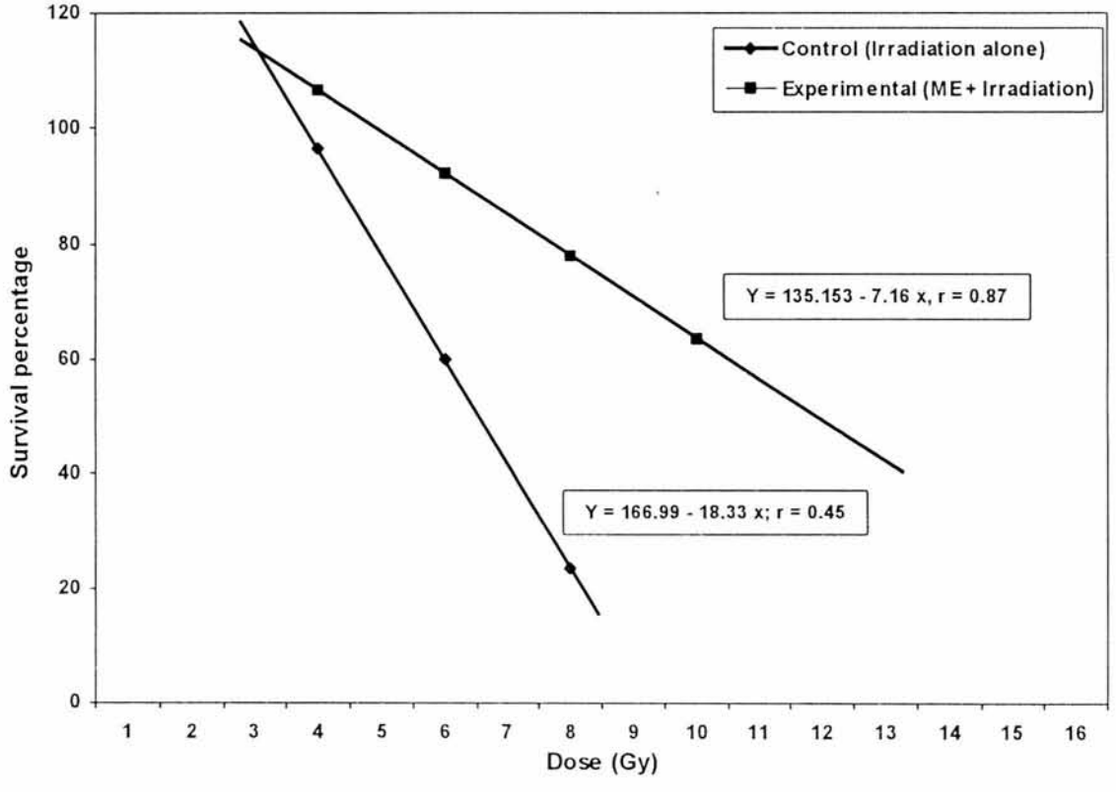

Fig. 2 — Survival dose-response curves for determination of $LD_{50/30}$ (Survival data collected for four radiation doses and were calculated by regression analysis)

Mentha treated animals. However, a significant decrease in GSH was recorded in control animals (irradiation alone). *Mentha* pretreated irradiated animals showed a significant increase in GSH content (blood as well as liver) with respect to control, but such values remained below normal. An increase in TBARS level in liver and serum was evident in control animals. Although, no significant difference was noticed in such levels in normal and *Mentha* treated unirradiated animals, but a significant decrease was registered in *Mentha* pretreated irradiated animals (Figs 7,8).

Discussion

In the present investigation, it has been observed that *Mentha* pretreated irradiated animals did not show any mortality till day 7 at all radiation doses studied. Thus, *Mentha* pretreatment showed protection against radiation-induced gastro-intestinal damage; thereby enhanced survival of mice is observed. It was also observed that pretreatment of *Mentha* extract protects mouse jejunum against the radiation induced reduction in villus height, total cells and mitotic figures/crypt section. *Mentha* pretreatment also protects against radiation induced increase in goblet cells/villus section and dead cells/crypt section in jejunum of mice[11]. It has been observed that *Mentha* pretreated animals showed only 18 and 42 % mortality as compared to 100% mortality in control (irradiation alone) at 8 and 10 Gy respectively. These results indicate that *Mentha* pretreatment have also provided protection against the hematopoietic death. It was evident from our earlier study that *Mentha* administration elevated the counts of endogenous spleen colonies and spleen weight significantly[9]. The enhanced survivability observed in *Mentha* pretreated mice were probably due to accelerated hematopoietic regeneration.

In the present study, the frequency of aberrant cells; chromosome breaks, centric rings, dicentrics, exchanges and acentric fragments significantly increased from 6 hr and reached to maximum at 12 hr in animals exposed to 8 Gy gamma radiation. Exposure to radiation is known to produce a significant increase in the per cent aberrant metaphases as well as in the different aberrations. Damage to the chromosomes is manifested as breaks and fragments, which appear as micronuclei in the rapidly proliferating cells[17]. Enhancement in the frequency of micronuclei and chro-

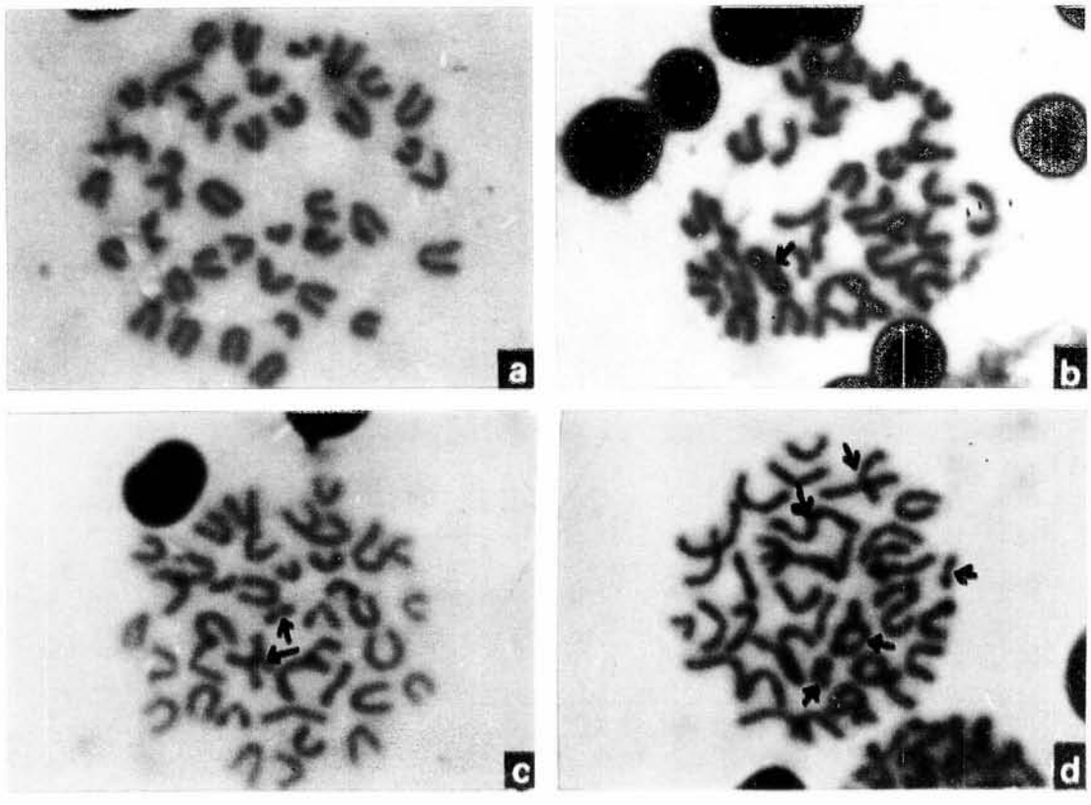

Fig. 3 — Metaphase chromosome preparations from bone marrow of Swiss albino mice (×1000), (a) normal; (b) dicentric ring; (c) exchange, chromosome break; (d) exchanges, rings, fragments

mosomal aberrations has been reported earlier in the bone marrow of irradiated mice[18,19].

Ionizing radiation is a highly efficient cytotoxic agent. An X-ray dose of 1.5 Gy produces approximately 10^{-6} *M* radicals in cells[20]. DNA is the critical target for cell killing by ionizing radiation, and there is growing evidence that the particular damage responsible are DNA double-strand lesions, such as DSB[21], while damage to other biological molecules does occur and is potentially cytotoxic. It has been recognized that structural aberrations can be induced in chromosomes by radiation at any stage of their mitotic cycle. When cells are irradiated just as they enter division, there is apparently some change in the surface properties of the chromosomes, which cause them to adhere to each other when they happen to

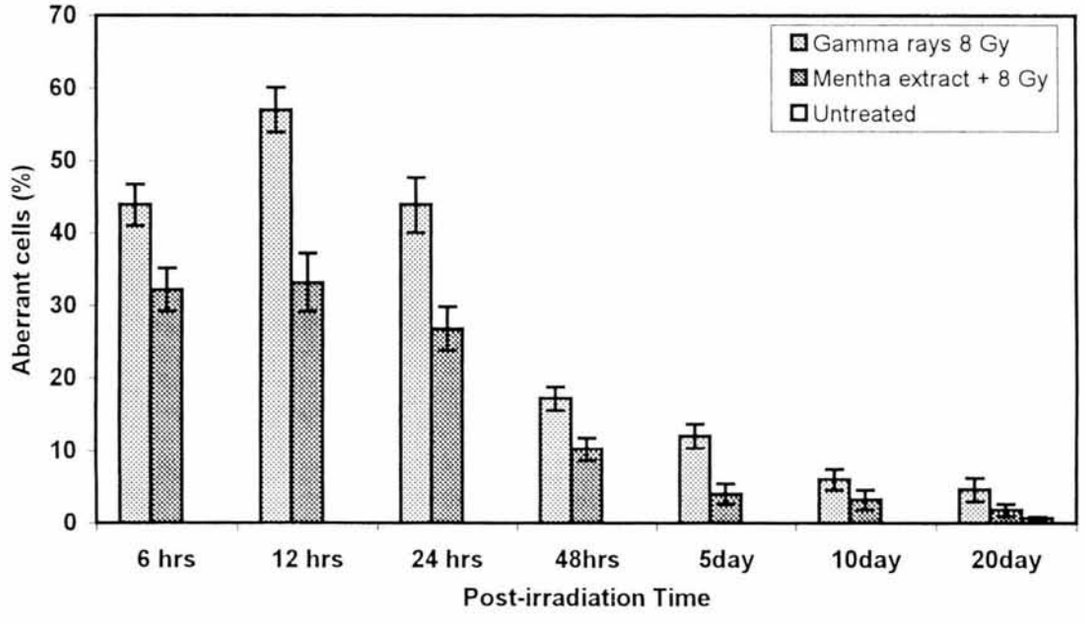

Fig. 4 — Aberrant cells (%) in the bone marrow of mice with or without *Mentha* treatment and exposed to 8 Gy gamma radiation

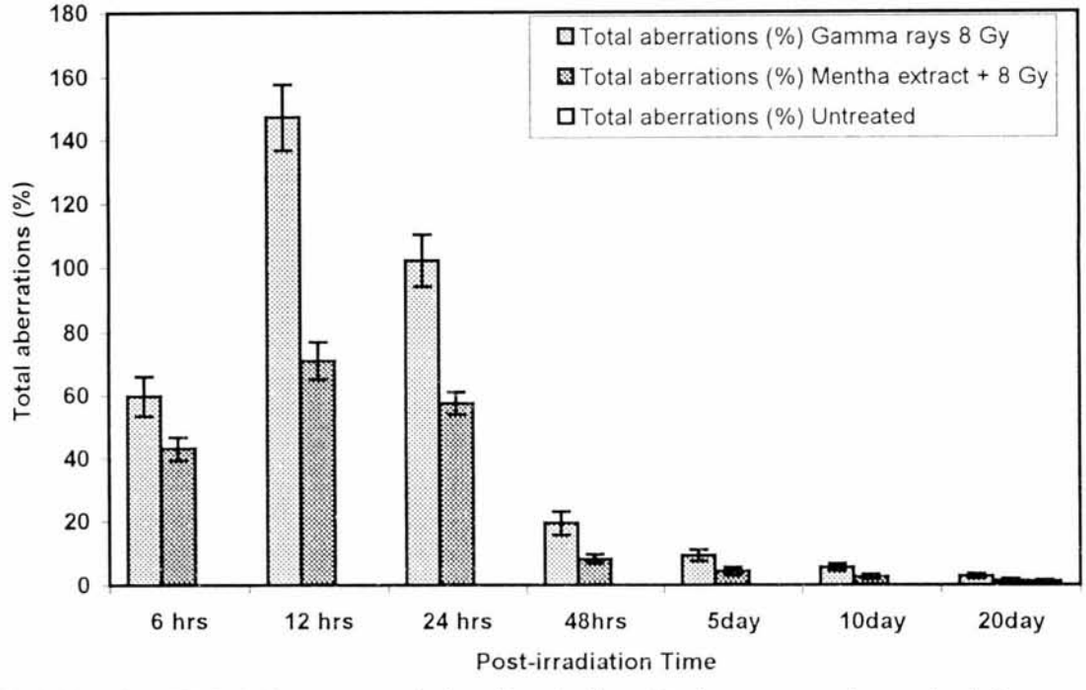

Fig. 5 — Total aberrations (%) in the bone marrow of mice with and without *Mentha* treatment and exposed to 8 Gy gamma radiation

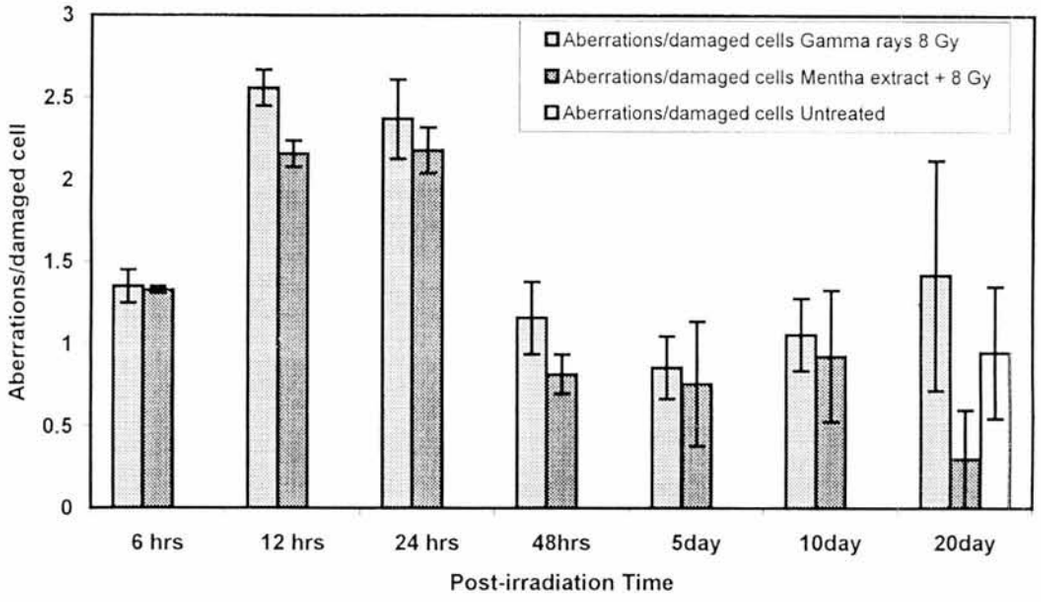

Fig. 6 — Aberrations/damaged cell in the bone marrow of mice with or without *Mentha* treatment and exposed to 8 Gy gamma radiation

Table 1 — Frequency of chromosomal aberrations in the bone marrow of Swiss albino mice with or without mentha treatment and exposed to 8 Gy gamma radiation

Aberrations per 100 cells	Group	Post-irradiation time						
		6 hr	12 hr	24 hr	48 hr	5 days	10 days	20 days
Chromatid breaks (0.16 ± 0.06)	Gamma rays (8 Gy)	5.8 ± 1.05[c]	11.90 ± 1.34[c]	8.20 ± 1.22[c]	1.40 ± 0.19[c]	0.92 ± 0.28[a]	0.40 ± 0.14	0.16 ± 0.07
	Mentha extract + gamma rays	4.08 ± 0.54	3.94 ± 1.18[b]	2.46 ± 0.63[b]	0.76 ± 0.18[a]	0.66 ± 0.24	0.36 ± 0.14	0.00
Chromosome breaks (0.00)	Gamma rays (8 Gy)	2.09 ± 0.33[c]	5.79 ± 0.79[c]	4.27 ± 0.42[c]	1.79 ± 0.42[b]	0.63 ± 0.15[b]	0.00	0.00
	Mentha extract + gamma rays	1.14 ± 0.23[a]	2.70 ± 0.33[b]	2.46 ± 0.52[a]	0.90 ± 0.19	0.22 ± 0.07[a]	0.00	0.00
Centric rings (0.00)	Gamma rays (8 Gy)	1.72 ± 0.27[c]	1.70 ± 0.32[c]	2.56 ± 0.29[c]	0.43 ± 0.15[a]	0.00	0.00	0.00
	Mentha extract + gamma rays	1.16 ± 0.28	1.14 ± 0.18	1.91 ± 0.14	0.18 ± 0.09	0.00	0.00	0.00
Dicentrics (0.00)	Gamma rays (8 Gy)	1.82 ± 0.35[c]	3.87 ± 0.66[c]	1.25 ± 0.30[b]	0.34 ± 0.06[c]	0.20 ± 0.08[a]	0.00	0.00
	Mentha extract + gamma rays	1.00 ± 0.13	1.80 ± 0.25[a]	0.29 ± 0.10[a]	0.10 ± 0.03[a]	0.00	0.00	0.00
Exchanges (0.00)	Gamma rays (8 Gy)	1.80 ± 0.43[b]	2.23 ± 0.29[c]	0.62 ± 0.10[c]	0.26 ± 0.06[b]	0.00	0.00	0.00
	Mentha extract + gamma rays	1.16 ± 0.33	1.01 ± 0.12[b]	0.32 ± 0.06[a]	0.06 ± 0.02[a]	0.00	0.00	0.00
Fragments (1.10±0.37)	Gamma rays (8 Gy)	46.6 ± 5.19[c]	121.6 ± 9.01[c]	85.2 ± 7.62[c]	15.2 ± 3.61[b]	7.6 ± 1.72[b]	5.2 ± 1.06[b]	2.6 ± 0.74
	Mentha extract + gamma rays	34.6 ± 3.28[c]	60.4 ± 5.08[b]	50.0 ± 3.93[a]	6.0 ± 1.41[a]	3.4 ± 1.32	2.2 ± 0.73	1.4 ± 0.60
Total aberrations (0.95±0.40)	Gamma rays (8 Gy)	59.86 ± 6.26[c]	147.11 ± 10.38[c]	102.11 ± 8.08[c]	19.42 ± 3.75[b]	9.35 ± 1.85[b]	5.60 ± 1.14[b]	2.76 ± 0.74
	Mentha extract + gamma rays	43.14 ± 3.62[a]	71.01 ± 6.02[c]	57.45 ± 3.60[b]	8.21 ± 1.51[a]	4.28 ± 1.37	2.56 ± 0.74	1.40 ± 0.60

Values in parentheses indicate values for untreated. 400 metaphases were scored/animal. Significance levels: [a]$P<0.05$; [b]$P<0.01$;; [c]$P<0.001$

Statistical comparison: Gamma rays (8Gy) vs Untreated; *Mentha* extract + gamma rays vs Gamma rays (8Gy)

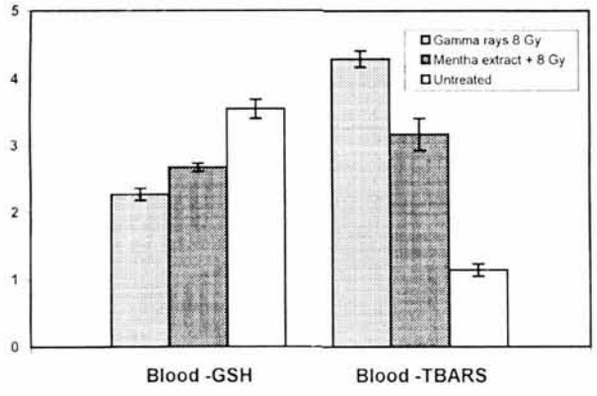

Fig. 7 — Blood GSH (μg/ml) and LPO (nmole/ml) levels of mice with or without *Mentha* treatment and exposed to 8 Gy gamma radiation

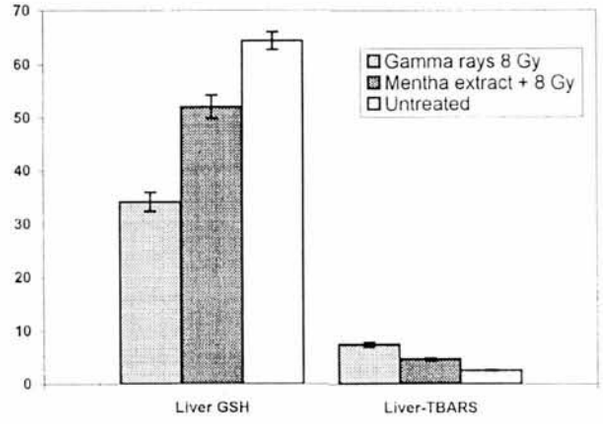

Fig. 8 — Liver GSH (μmole/g) and LPO (nmole/mg) levels of mice with or without *Mentha* treatment and exposed to 8 Gy gamma radiation

touch. This stickiness has been attributed to a partial dissociation of the nucleoproteins and an alteration in their pattern of organization[22]. Natarajan *et al.*[23] and Bryant[24] suggested that double strand breaks (dsb) are mainly responsible for the formation of chromosomal aberrations.

It has been observed that pretreatment with *Mentha* extract was effective in protecting the chromosomal damage against irradiation, as is evidenced by a significantly lower levels of chromatid breaks, chromosome breaks, centric rings, dicentrics, exchanges, fragments and total aberrations. It is well known that free radicals generated during radiolysis of water play the most significant role in the indirect biological damage induced by ionizing radiation[25]. The GSH/GST detoxification system is an important part of cellular defense against a large array of injurious agents. GSH offers protection against oxygen derived free radicals and cellular lethality following exposure to ionizing radiation[26]. Under normal conditions the inherent defense system including glutathione and the antioxidant enzymes, protects against the oxidative damage. GSH is a versatile protector and executes its radioprotective function through free radical scavenging, restoration of the damaged molecule by hydrogen donation, reduction of peroxides and maintenance of protein thiols in the reduced state[20].

The present study demonstrates a significant reduction in liver and blood GSH, following exposure. This could be due to the enhanced utilization of the antioxidant system as an attempt to detoxify the free radicals generated by radiation. Oral administration of *Mentha* extract did not significantly influence the endogenous GSH level either in liver or blood, but its presence while radiation exposure protects the endogenous GSH depletion due to irradiation. The lower depletion of liver and blood GSH in the *Mentha* pretreated irradiated animals could be due to the higher availability of GSH, which increases the ability to cope up with the free radicals produced by radiation. The increased GSH level suggests that protection by *Mentha* may be mediated through the modulation of cellular antioxidant levels. Ochi[27] have reported that chromosomal aberrations were repaired only in GSH positive cells. Thus it is possible that the elevated levels of GSH in *Mentha* pretreated irradiated animals may be able to enhance the repair of dsb, hence lower frequency of chromosomal aberrations was evident in this group.

The basic effect of radiation on cellular membranes is believed to be the peroxidation of membrane lipids. Radiolytic products, including hydroxyl and hydroperoxyl radicals can initiate lipid peroxidation[28]. In the present study, it was observed that, although *Mentha* treatment did not significantly alter the lipid peroxidation level in unirradiated animals, it significantly lowered the radiation induced lipid peroxidation in terms of malondialdehyde. Inhibition of lipid peroxidation in biomembranes can be caused by antioxidants [29,30]. It has been shown that more α-tocopherol is needed in the membranes to protect polyunsaturated fatty acids (PUFA) against radiation induced lipid peroxidation when low dose-rates are applied[31].

Several mechanisms, including a potent antioxidant activity, immune response and enhanced recovery of bone marrow have been suggested for radioprotection by vitamin E[32]. In the present study, it was observed that *Mentha* pretreated irradiated animals exhibited a significant increase in GSH and decrease in LPO

level. *Mentha* extract has been shown to have antioxidant and antiperoxidant properties due to the presence of eugenol, caffeic acid, rosmarinic acid and α-tocopherol[2-4]. Vokovic-Gacis and Simic[7] showed that extracts of mint (*Mentha*) could enhance error-free repair of damage and, hence, could be antimutagenic. Samman *et al.*[8] reported that *Mentha piperita* has a chemopreventive effect against shamma induced carcinogenesis, which could be due to antimutagenic properties. A combination of antioxidative and antimutagenic activities via modulation of DNA repair processes may be held responsible for the radioprotective effects of *Mentha piperita* (Linn.) leaf extract.

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