Radioprotection of Swiss Albino Mice by Plant Extract *Mentha piperita* (Linn.)

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***Mentha piperita* (Linn.)/CFU-S/$LD_{50/30}$/GSH/LPO/Serum phosphatases activity/Gamma radiation.**

The oral administration of *Mentha* extract (ME) before exposure to gamma radiation was found to be effective in increasing the frequency of radiation-induced endogenous spleen colonies. A significant increase in the weight of the spleen was observed in animals of the *Mentha* and radiation combined group in comparison to the irradiation-alone group on day 10 of postirradiation. Furthermore, a significant increase in the body weight of animals in the *Mentha* and radiation combined group was observed in all the radiation doses studied. A regression analysis of survival data yielded $LD_{50/30}$ as 6.48 ± 0.07 and 11.59 ± 0.21 Gy for the irradiation-alone and the *Mentha* and radiation combined group, respectively, and produced a dose reduction factor (DRF) of 1.78. Significant increases in total erythrocyte and leucocyte counts, hemoglobin concentration, and hematocrit values were observed in the animals of the *Mentha* and radiation combined group in comparison to the hematological values observed in the irradiation-alone group at all radiation doses studied (6, 8, and 10 Gy). A dose-dependent decrease in reduced glutathione (GSH) content and an increase in lipid peroxidation (LPO) levels were observed in control animals. However, the animals of the *Mentha* and radiation combined group exhibited a significant increase in GSH content and a decrease in LPO level, but the values remained below normal. A significant increase in the serum alkaline phosphatase activity was observed in the animals of the *Mentha* and radiation combined group during the entire period of study, and normal range was evident at 24 h (6 Gy) and day 5 (8 Gy). However, this level could not be restored even at day 30 in 10 Gy exposed animals. Measured acid phosphatase activity in the animals of the *Mentha* and radiation combined group was found to be significantly lower than the respective controls and attained normal value at day 5 (6 and 8 Gy) and day 20 (10 Gy).

INTRODUCTION

Interest has recently increased in the development of potential drugs of plant origin for the modification of radiation effects. Plant extract such as garlic, ginseng, *Aloe vera*, *podophyllum*, *ocimum*, *spirulina* and herbal drug preparations such as Liv.52 and rasayanas have been found to have radioprotective effects in mammals[1–9]. Plant products appear to have an advantage over the synthetic compounds in terms of low/no toxicity at the effective dose with minimum/no side effects.

Mentha piperita (Linn.), commonly called peppermint (Labiatae), is considered an aromatic, stimulant, and carminative. It is being used for allaying nausea, flatulence, and vomiting[10]. *Mentha* extract has been shown to have antioxidant and antiperoxidant properties because of the presence of eugenol, caffeic acid, rosmarinic acid, and α-tocopherol[11-13]. The aqueous extract has also been screened for antibacterial activity against *Pseudomonas solanacearum*[14]. The present study deals with the radioprotective activity of the aqueous extract of *Mentha piperita* (Linn.) in terms of body weight, spleen colonies, and survivability of Swiss albino mice.

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MATERIALS AND METHODS

Animals

Male Swiss albino mice (*Mus musculus*), 6–8 weeks old with 25 ± 2 gm body weight, from an inbred colony (obtained from Hamadard University, Delhi) were used for the present study. The animals were maintained under controlled conditions of temperature and light in an animal house and were provided standard mice feed (procured from Hindustan Lever's Ltd., Delhi) and water *ad libitum*.

Irradiation

A cobalt teletherapy unit (ATC-C9) at the Cancer Treatment

Centre, Radiotherapy Department, SMS Medical College & Hospital, Jaipur, was used for irradiation. Unanesthetized animals were restrained in well-ventilated Perspex boxes and the whole body exposed to gamma radiation at a distance (SSD) of 77.5 cm from the source to deliver the dose rate of 1.59 Gy/min.

Mentha extract

Plant material [*Mentha piperita* Linn.] was collected locally and identified, and the specimen was placed at Herbarium, Department of Botany, University of Rajasthan, Jaipur. The voucher number is RUBL-19443. Fresh leaves (100 gm) of *Mentha piperita* (Linn.) were washed, air dried, powdered, and extracted with 1,500 ml of double-distilled water (DDW) by refluxing for 36 h (3 × 12 h) at 80°C. The extract thus obtained was vacuum evaporated to make it into powder form. The extract was redissolved in DDW just before the oral administration. For the various concentrations, a known amount of ME was suspended in DDW, and 0.1 ml of ME suspension was given to each mouse by oral gavage.

Experimental design

*The determination of optimum dose of Mentha extract against radiation:*The mice were divided into six groups of 10 animals each and given *Mentha* orally (0.125, 0.25, 0.5, 1, 2, and 4 gm/kg body weight/day) for three consecutive days. Thirty minutes after the final administration, the animals were exposed whole-body to 8 Gy gamma radiation. All animals were observed for 30 days for any sign of radiation sickness, morbidity, behavioral toxicity, or mortality. The optimum dose thus obtained was used for further investigation.

Modification of radiation response: The animals selected for this study were divided into two groups. Those of one group were administered *Mentha* orally (1gm/kg body weight/day) for three consecutive days, and the control group received DDW (volume equal to ME). After 30 minutes of these treatments on the day, the animals of both groups were exposed to different doses of gamma radiation (4, 6, 8, and 10 Gy).

Body weight: The general condition of the mice was observed daily and recorded through measurement of body weights. The percent of change in each group was recorded daily by dividing the average weight of mice surviving on a given day by the average weight of the same mice treated on the first day.

Endogenous spleen colony assay

The endogenous spleen colony assay was done according to the method of Till and McCulloch[15]. Endogenous spleen colony forming units (CFU-S) were determined on day 10 after irradiation. The animals were sacrificed by cervical dislocation, and their spleens were removed, weighed, and fixed in Bouin's fixative. Grossly visible nodules on the spleen surfaces were counted by the naked eye.

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 percentage of mice surviving 30 days of exposure against each radiation dose was used to construct survival–dose response curves.

Hematological study

Blood was collected from the tail vein of each animal in a vial containing 0.5 M EDTA. Peripheral blood counts (RBC/WBC), hemoglobin (Hb) concentration, and hematocrit (Hct) percentage were determined at 6, 12, 24, 48 h and 5, 10, 20, and 30 days postirradiation by adopting standard procedures.

Biochemical study

(1) Reduced glutathione (GSH) assay: The hepatic level of reduced glutathione (GSH) was determined by the method described by Moron *et al.*[16]. GSH content in blood was measured spectrophotometrically by using Ellman's reagent (DTNB) as a coloring reagent, following the method described by Beutler *et al.*[17]. The absorbance was read at 412 nm with a UV-VIS Systronics spectrophotometer.

(2) Lipid peroxidation assay: The lipid peroxidation level in liver and serum was measured in terms of Thiobarbituric Acid Reactive Substances [TBARS] by the method of Ohkhawa *et al.*[18]. The absorbance was read at 532 nm.

(3) Serum phosphatases activity: Serum alkaline phosphatase activity was measured by Kind and King's method[19]. Acid phosphatase activity was determined by King's method[19] by using commercially available kits.

Statistical analysis

The results obtained were expressed as mean ± SE. A Student's *t* test was used to make a statistical comparison between the groups. A statistical comparison was completed with the irradiation-alone group vs. the normal and irradiation-alone group vs. the *Mentha* and radiation combined group. The significance levels were set at $P < 0.05$, $P < 0.005$, and $P < 0.001$. Regression analysis was done to obtain $LD_{50/30}$ values and to determine the dose reduction factor (DRF)

RESULTS

No toxic effects were observed in terms of sickness, body weight, urination and defecation pattern, and mortality in animals treated with ME alone. The optimum dose of ME exhibiting maximum radioprotection was found to be 1 gm/kg body weight/day for three consecutive days before irradiation (8 Gy). The animals pretreated with 0.5, 1, 2, and 4 (gm/kg body weight/day) showed 70, 82, 60, and 40% survival, respectively, against 8 Gy gamma radiation. All animals pretreated with 0.125 and 0.25 (gm/kg body weight/day) ME died within 11 days of irradiation (Fig. 1). Therefore 1 gm/kg body weight/day ME was used for the detailed investigation.

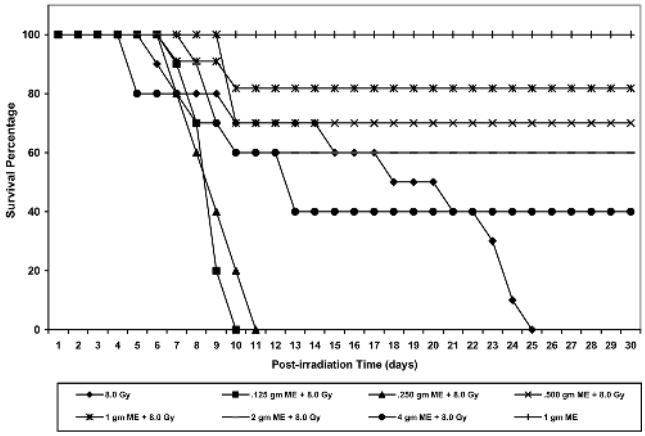

Fig. 1. Thirty-day survival of mice pretreated with different doses of ME and exposed to 8 Gy gamma radiation.

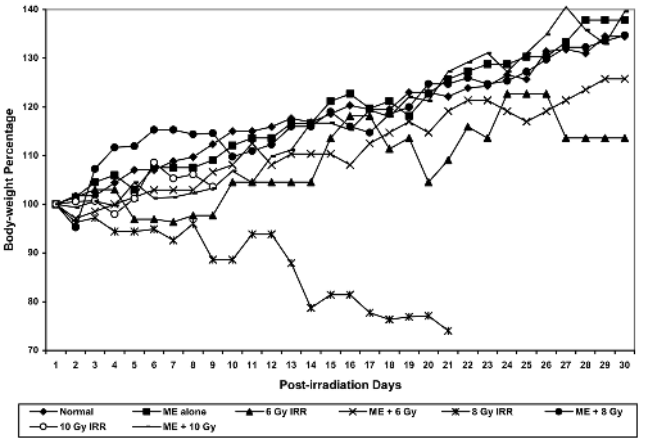

Fig. 2. Body weight change (as a percentage of average body weight on the first day of treatment) of Swiss albino mice with or without ME treatment and/or exposed to gamma radiation.

During the experimental period, Sham-irradiated animals showed a consistent weight gain by reaching almost 34.50% higher than the initial weight at day 30. A biphasic, significant loss in the body weight of animals of the irradiation-alone group was observed at all radiation doses studied (6, 8, and 10 Gy). The animals of the *Mentha* and radiation-combined group showed a more significant increase in body weight than the respective controls did at each radiation dose, and they exhibited a progressive weight gain toward normal by day 30 of postirradiation (Fig. 2).

The protective effect of ME against radiation injury to hematopoietic tissues was assessed by the endogenous spleen colony assay and spleen weight. A significant increase was found in the number of spleen colonies in the *Mentha* and radiation-combined group (1.80 ± 0.59, 11.80 ± 0.59, 15.20 ± 0.91, and 19.20 ± 0.71 at 4, 6, 8, and 10 Gy, respectively). A significant loss in spleen weight at day 10 following irradiation was observed in the animals of the irradiation-alone group. On the contrary, a significant increase in spleen weight was noticed in animals of the *Mentha* and radiation-combined group (Table 1).

Table 1. Spleen response on day 10 after whole-body irradiation to different doses of gamma radiation with or without ME treatment in Swiss albino mice.

Radiation dose (whole-body)	Group	Spleen weight (mg)	No. of macroscopic colonies
0 Gy	Normal	41.20 ± 0.91	–
4 Gy	Control	34.80 ± 0.95[b]	–
	Experimental	41.40 ± 0.77[c]	1.80 ± 0.59
6 Gy	Control	21.20 ± 0.91[c]	0.80 ± 0.33
	Experimental	35.20 ± 0.76[c]	11.80 ± 0.59
8 Gy	Control	20.60 ± 1.04[c]	–
	Experimental	39.00 ± 1.19[c]	15.20 ± 0.91
10 Gy	Control	N.S.	N.S.
	Experimental	35.40 ± 0.66[c]	19.20 ± 0.71

In the present study, a dose-dependent survivability of mice was observed in both groups. However, the survival percentage in the experimental group was higher than in the irradiation-alone group. In a later group, no mortality was observed at 4 Gy, but a third (33%) of the total animals died within 30 days after exposure to 6 Gy gamma radiation, whereas no animal could survive until day 30 when exposed to 8 and 10 Gy gamma radiation. The first deaths were recorded on days 7, 6, and 4 at 6, 8, and 10 Gy doses, respectively. At 8 Gy radiation dose, 20% of the animals died within a week, and 100% of the animals had died by day 25. Exposure to 10 Gy gamma radiation resulted in a 54% mortality of the mice by day 7, whereas 100% mortality had occurred within 10 days.

In the present investigation, it was observed that the pretreatment of ME enhanced the survival percentage of mice exposed to different doses of gamma radiation. ME pretreatment 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% of deaths occurred from day 7 to day 10 (Fig. 3). Radiation dose-response curves for mice with or without a pretreatment of ME are shown in Fig. 4. The $LD_{50/30}$ for the irradiation-alone group and the *Mentha* and radiation combined group of animals was computed as 6.48 ± 0.07 and 11.59 ± 0.21 Gy, respectively. In this way, on the basis of $LD_{50/30}$ survivability, ME pretreatment produced a dose-reduction factor (DRF) of 1.78.

Animals treated with ME alone showed no significant alteration in hematological values in comparison to the normal animals. A significant decrease in hematological values (RBCs, WBCs, Hb, and Hct) of the irradiation-alone group of animals, compared to normal animals, was observed at all radiation doses studied (6, 8, and 10 Gy). At 6 Gy, however, hematological values showed recovery by day 30 of postirradiation. In the *Mentha* and radiation combined group, a significant increase in the total number of erythrocytes was observed in comparison to the respective controls at each interval. The maximum decrease in the number of these cells was observed at 24 h (6 and 8 Gy) and

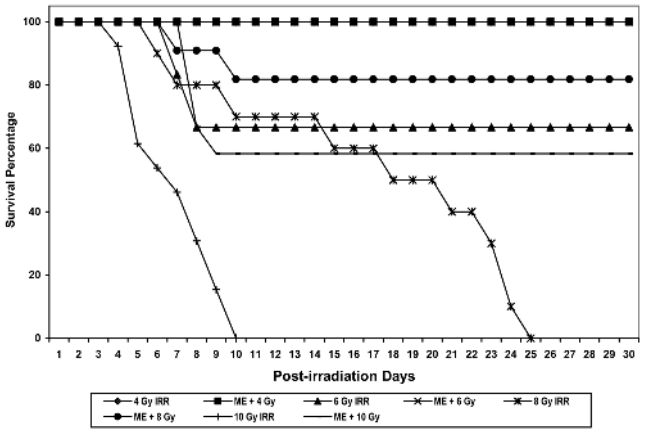

Fig. 3. Thirty-day survival of mice with or without ME treatment after exposure to different doses of gamma radiation.

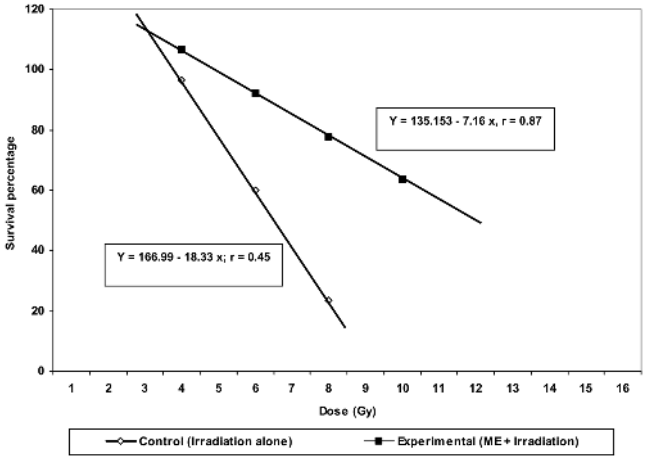

Fig. 4. Survival dose-response curves for the determination of $LD_{50/30}$ (survival data collected for four radiation doses and calculated by regression analysis).

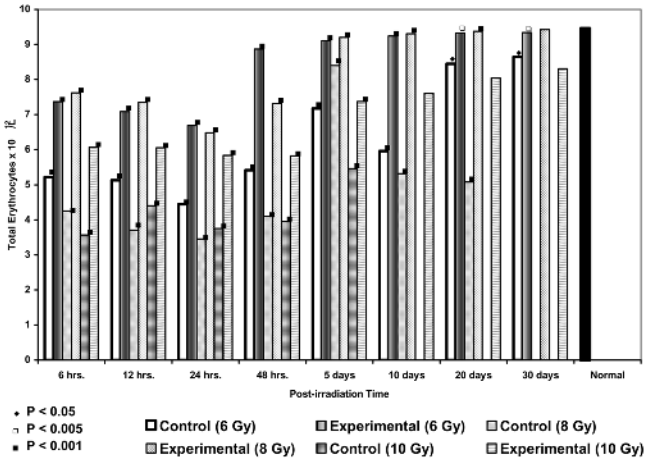

Fig. 5. Variation in the total erythrocyte counts of mice with and without ME treatment and exposed to different doses of gamma radiation.

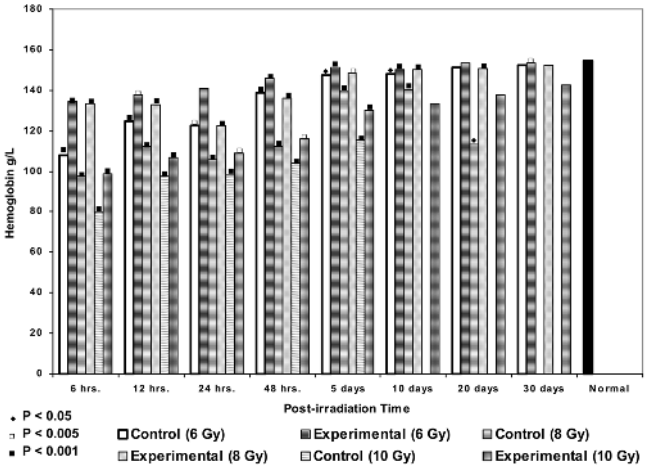

Fig. 6. Variation in the hemoglobin concentration of mice with and without ME treatment and exposed to different doses of gamma radiation.

48 h (10 Gy). The number of erythrocytes increased from day 5, and normalcy was restored by day 30 of postirradiation at all three radiation doses studied (Fig. 5). ME pretreatment also showed a significantly increased concentration of hemoglobin over the respective controls, and normal value was evident on day 5 (6 Gy) and day 30 (8 Gy), but the value could not reach normal even on day 30 at 10 Gy (Fig. 6). Hematocrit values showed a consistent recovery from 6 h onward in the *Mentha* pretreated irradiated animals and reached normal on day 5 (6 Gy) and day 30 (8 Gy). At 10 Gy, normal value could not be restored even at day 30 (Fig. 7). Total leucocyte count (WBC) was observed significantly above respective controls at all three radiation dose levels studied in animals of the *Mentha* and radiation combined group and achieved normal value on day 30 (6 Gy) and day 20 (8 Gy). At 10 Gy, the values remained significantly below those of the normal animals (Fig. 8). Thus ME pretreatment showed protection of the hematological constituents against gamma irradiation.

No significant variation in the hepatic or the blood GSH contents was observed between normal and ME-alone treated animals. However, a dose-dependent decrease in GSH was recorded in animals of the irradiation-alone group. This decrease was more at a higher dose of radiation (10 Gy) than the lower one (8 and 6 Gy). The animals of the *Mentha* and radiation combined group showed a significant increase in GSH content (blood and liver) with respect to control, but these values remained below those of the normal group (Table 2).

A dose-dependent increase in TBARS level in liver and serum was evident in the irradiation-alone group (Table 2), though, no significant difference was noticed in these levels in normal and ME-treated animals. A significant decrease was registered in animals of the *Mentha* and radiation combined group, however.

In animals of the *Mentha* and radiation combined group, a greater significant increase in the serum alkaline phosphatase activity was noted than in the corresponding controls during the

Table 2. Reduced glutathione (GSH) and lipid peroxidation (LPO) levels in the blood and liver of Swiss albino mice with and without ME treatment after exposure to different doses of gamma radiation.

Treatment	Blood GSH (µg/ml)	TBARS (nmol/ml)	Liver GSH (µmol/gm)	TBARS (nmol/mg)
Normal	3.545 ± 0.14	1.14 ± 0.09	64.39 ± 1.63	2.60 ± 0.10
ME	3.653 ± 0.13	1.01 ± 0.12	64.81 ± 3.10	2.17 ± 0.10[a]
6 Gy (control)	2.698 ± 0.08[c]	3.60 ± 0.18[c]	40.59 ± 1.60[c]	6.68 ± 0.26[c]
ME + 6 Gy (experimental)	2.915 ± 0.08	2.44 ± 0.17[b]	53.12 ± 1.71[c]	3.57 ± 0.20[c]
8 Gy (control)	2.269 ± 0.09[c]	4.29 ± 0.12[c]	34.12 ± 1.77[c]	7.51 ± 0.45[c]
ME + 8 Gy (Experimental)	2.668 ± 0.06[b]	3.17 ± 0.24[b]	52.06 ± 2.18[c]	4.74 ± 0.21[c]
10 Gy (control)	2.044 ± 0.03[b]	5.08 ± 0.21[c]	27.83 ± 1.39[c]	8.52 ± 0.27[c]
ME + 10 Gy (experimental)	2.524 ± 0.07[b]	3.62 ± 0.18[c]	47.38 ± 1.76[c]	5.24 ± 0.26[c]

Normal = No treatment. Significance level: [a]$P < 0.05$; [b]$P < 0.005$; $P < 0.001$. ME = (1,000 mg/kg body wt./day) for 3 days. Statistical comparison: control vs. normal: control = irradiation alone; experimental vs. control: experimental = *Mentha* + irradiation. Each value represent mean ± SEM.

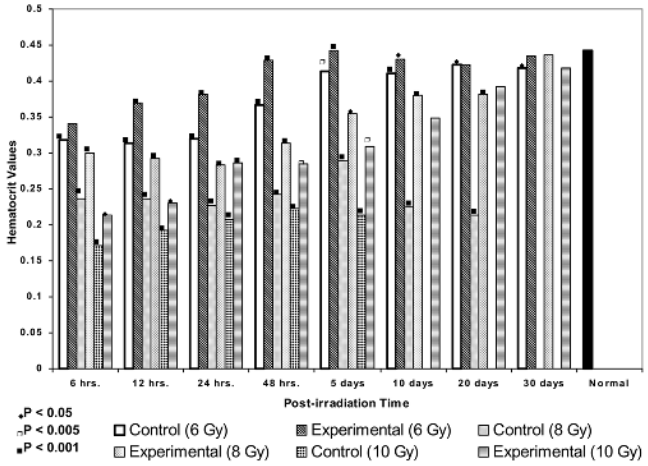

Fig. 7. Variation in the hematocrit values of mice with and without ME treatment and exposed to different doses of gamma radiation.

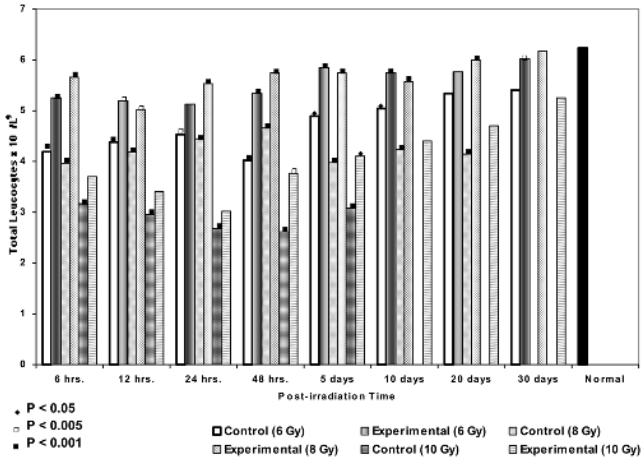

Fig. 8. Variation in the total leucocyte counts of mice with and without ME treatment and exposed to different doses of gamma radiation.

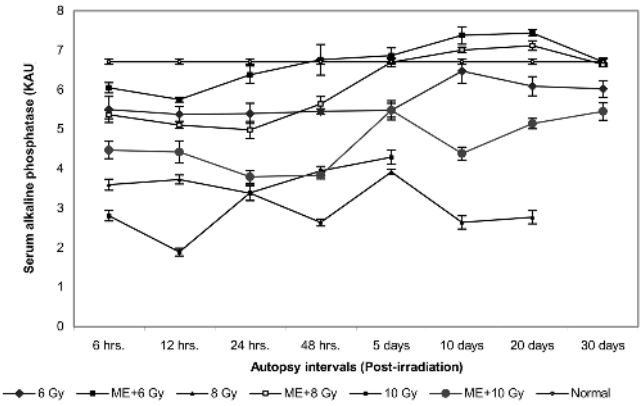

Fig. 9. Variation in the alkaline phosphatase activity of mice with and without ME treatment and exposed to different doses of gamma radiation.

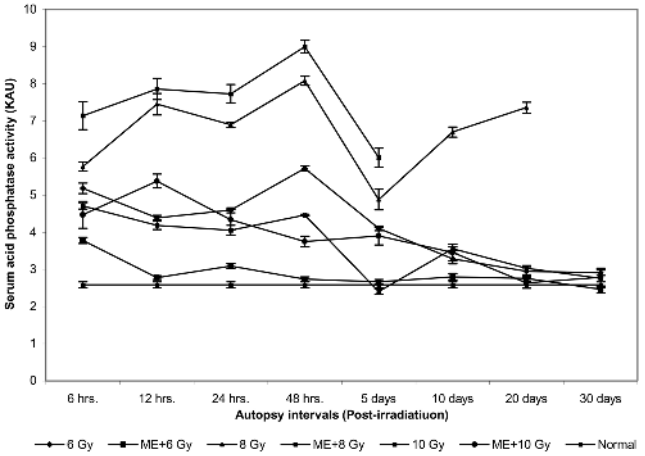

Fig. 10. Variation in the acid phosphatase activity of mice with and without ME treatment and exposed to different doses of gamma radiation.

entire period of study, and a normal range was evident at 24 h (6 Gy) and day 5 (8 Gy). However, this level could not be restored even at day 30 in the 10 Gy exposed group (Fig. 9). Serum acid phosphatase activity in animals of the *Mentha* and radiation combined group was measured significantly lower than the respective controls and attained the normal range at day 5 (6 and 8 Gy) and day 20 (10 Gy). Although the acid phosphatase values showed more significant decrease than the control values did, they remained higher than normal. Thus acid phosphatase activity showed an increase over the normal animals in the *Mentha* and radiation combined group (Fig. 10).

DISCUSSION

The results from the present study indicate that the pretreatment of ME protects the hematopoietic tissues in mice from the lethal effects of ionizing radiation. The radioprotective effect of ME was demonstrated by determining the $LD_{50/30}$ (DRF = 1.78) and endogenous spleen colony assay. A significant radioprotection was achieved when ME was given orally (1 gm/kg body weight/day) for 3 consecutive days before irradiation.

In the present study, maximum radioprotection (82% survival) was observed at 1 gm/kg body weight/day ME for three consecutive days before irradiation. However, at higher concentrations (2 and 4 gm/kg body weight/day) the protection was less in comparison to 1 gm/kg body weight/day (60 and 40% survival). A similar modulation of radiation/immune effects at low doses, instead of at the higher nontoxic doses, has been reported on MPG (2-mercaptopropionylglycine), ginseng extract, glucan, bacterial preparation, and serum thymic factor (FTS). It has also been reported that ginseng extract, glucan, bacterial preparation, and serum thymic factor (FTS) mediate radioprotection through the enhancement of the production of various cytokines in irradiated animals[39–46]. FTS at higher concentration, however, is not effective. It was suggested that the ineffectiveness of high doses of FTS was not attributable to its toxicity, but it may be due to negative feedback reaction or to the down-regulation of FTS receptor in target cells[46]. Similarly, in the present study it is postulated that the less radioprotection at higher doses may be due to the negative feedback reaction or to the down-regulation of radioprotective cytokines receptors in target cells. The radiosensitizing effect of low concentrations of ME at both 0.125 and 0.25 gm on gamma radiation could not be explained at the present time; however, we are looking into the mechanisms of the radiosensitizing effect of ME.

It has been evident that damage to the hematopoietic system is a major factor in mortality following acute radiation exposure[20]. In the radiation dose range studied, hematopoietic gastrointestinal damage may contribute to mortality. In the present investigation, it was observed that 17, 20, and 54% of the animals died within day 7 after exposure to 6, 8, and 10 Gy gamma radiation, respectively. Thirty-three percent mortality occurred in animals exposed to 6 Gy gamma radiation, whereas 100% mortality was observed in animals exposed to 8 Gy (within 25 days) and to 10 Gy (within 10 days). This mortality can be attributed to hematopoietic death because radiation damage to gastrointestinal epithelium cannot be expected to contribute to mortality in mice surviving more than 7 days after irradiation, since the restoration of the epithelium should be completed by this time[21]. In the present study, it has been observed that 20 and 54% of the animals died within day 7 at 8 and 10 Gy, respectively. The deaths that occurred in this period could have been due to the gastrointestinal syndrome. One common feature of radiation-induced gastrointestinal syndrome is a marked loss of water and electrolytes, which may contribute to the weight loss[22].

In the present study, a significant loss in body weight was evident in animals of the irradiation-alone group. The general pattern of biphasic weight response following whole-body irradiation agrees with those reported by Lamerton *et al.*[23]. The weight loss in the initial phase may probably be due to the gastrointestinal damage following irradiation[24,25]. The weight loss during the second phase was associated with the decrease in water intake[26]. A dose-dependent weight loss in the mouse has also been reported[27,28]. ME pretreated irradiated animals showed recovery in body weight from day 5 onward and reached normal by day 30 of postirradiation.

In the present investigation, it has been observed that animals of the *Mentha* and radiation combined group showed no mortality until day 7 in all radiation doses studied. Thus ME pretreatment showed protection against radiation-induced gastrointestinal damage; thereby an enhanced survival of mice is observed. It has been found that animals of the *Mentha* and radiation combined group showed only 18 and 42% mortality in comparison to 100% mortality in the animals of the irradiation-alone group at 8 and 10 Gy. These results indicate that ME pretreatment has also provided protection against hematopoietic death. It is evident from the present study that ME administration significantly elevated the counts of endogenous spleen colonies and spleen weight. The enhanced survivability observed in animals of the *Mentha* and radiation combined group was probably due to accelerated hematopoietic regeneration.

In the present study, a significant deficit in the hematological constituents of peripheral blood in animals of the irradiation-alone group was observed. The decrease in hematological constituents may be attributed to a direct damage by radiation dose[29]. Although 3 Gy total body dose is required to produce a detectable depression in total red blood cells, the whole-body irradiation of the moderate dose range (5-10 Gy) leads to a decreased concentration of all the cellular elements in blood. This can be due to a direct destruction of mature circulating cells, loss of cells from the circulation by hemorrhage, or leakage through capillary walls and a loss of the production of cells[20]. ME pretreatment showed a gradual recovery of hematological constituents in the peripheral blood of mice against radiation exposure (6, 8, and 10 Gy). Further, the hematological values restored normalcy at 48 h (6 Gy) and day 5 (8 Gy). Although at 10 Gy normalcy could not be achieved even at day 30, hematological values showed a gradual increase throughout the period

of study. This increase in the hematological constituents of peripheral blood in the animals of the *Mentha* and radiation combined group may possibly be due to accelerated hematopoietic regeneration.

It is well known that free radicals generated during the radiolysis of water play the most significant role in the indirect biological damage induced by ionizing radiation[30]. 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[31]. Under normal conditions the inherent defense system, including glutathione and the antioxidant enzymes, protects against oxidative damage. The present study demonstrates a significant reduction in liver and blood GSH following exposure. This could be due to an enhanced utilization of the antioxidant system as an attempt to detoxify the free radicals generated by radiation. The oral administration of ME to Swiss albino mice did not significantly influence the endogenous GSH level either in liver or in blood, but its presence during radiation exposure protects the endogenous GSH depletion resulting from irradiation. The lower depletion of liver and blood GSH in animals of the *Mentha* and radiation combined group could be due to the higher availability of GSH, which increases the ability to cope with the free radicals produced by radiation. The increased GSH level suggests that protection by ME may be mediated through the modulation of cellular antioxidant levels. 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[32].

The basic effect of radiation on cellular membranes is believed to be the peroxidation of membrane lipids. Lipid peroxidation can be initiated by radiolytic products, including hydroxyl and hydroperoxyl radicals[33]. In the present study, it was observed that although ME treatment did not significantly alter the lipid peroxidation level in unirradiated animals, ME pretreatment significantly lowered the radiation-induced lipid peroxidation in terms of malondialdehyde. The inhibition of lipid peroxidation in biomembranes can be caused by antioxidants. 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[34-36].

Serum acid phosphatase activity in animals of the *Mentha* and radiation combined group was found to be significantly lower than in animals of the irradiation-alone group and attained the normal value at day 5 (6 and 8 Gy) and day 20 (10 Gy). However, it remained significantly higher than normal at 24 h (6 Gy) and at 48 h (8 Gy) and day 10 (10 Gy). This suggests that ME may help in causing early recovery by the rapid removal of cellular debris from the tissue collected as a result of radiation damage. Acid phosphatase is a lysosomal enzyme that hydrolyses the ester linkage of phosphate ester and helps in the autolysis of the degenerated cells. On the other hand, alkaline phosphatase, a brush border enzyme, splits various phosphate esters in an alkaline medium and mediates membrane transport[37]. Thus an increase in these enzymes suggests that acid phosphatase helps in early recovery from radiation damage by removing debris, and alkaline phosphatase helps in stabilizing the membrane. The results from the present investigation indicate that ME pretreatment protects against radiation damage by inhibiting radiation-induced GSH depletion, decreasing LPO level, and increasing phosphatase activity in mice.

Several mechanisms, including a potent antioxidant activity, immune response, and enhanced recovery of bone marrow have been suggested for radioprotection[47]. It has also been reported that ME contains eugenol, caffeic acid, rosmarinic acid, and α-tocopherol, which have been shown to have antioxidant and antiperoxidant properties[11–13]. Shimoi *et al.*[38] concluded that plant flavonoids that show antioxidant activity *in vitro* also function as antioxidants *in vivo,* and their radioprotective effect may be attributed to their radical scavenging activity. The results from the present study suggest that hematopoietic stem cells can be protected from radiation-induced free radical damage by ME, which was evident in the increased number of radiation-induced spleen colonies (CFU-S) and hematological constituents in peripheral blood in animals of the *Mentha* and radiation combined group.

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