

Protection Against Radiation Induced Hematopoietic Damage in Bone Marrow of Swiss Albino Mice by *Mentha piperita* (Linn)

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Radiation protection/*Mentha piperita* Linn/Erythropoietin/Micronucleus frequencies/Bone marrow.

The protective effects of *Mentha piperita* (Linn) extract against radiation induced hematopoietic damage in bone marrow of Swiss albino mice have been studied. Mice were given either double distilled water or leaf extract of *M. piperita* orally (1 g/kg b.wt./day) once a day for three consecutive days, and after 30 min of treatments on the third day were exposed to 8 Gy gamma radiation. Mice were autopsied at 12, 24, 48 hrs and 5, 10 and 20 days post-irradiation to evaluate the percentage of bone marrow cells, frequency of micronuclei and erythropoietin level in serum. An exposure to gamma radiation resulted in a significant decline in the number of bone marrow cells such as leucoblasts, myelocytes, metamyelocytes, band/stab forms, polymorphs, pronormoblasts and normoblasts, lymphocytes, and megakaryocytes. Pretreatment with leaf extract of *M. piperita* followed by radiation exposure resulted in significant increases in the numbers of leucoblasts, myelocytes, metamyelocytes, band/stab forms, polymorphs, pronormoblasts and normoblasts, lymphocytes, and megakaryocytes in bone marrow as compared to the control group. Pretreatment with leaf extract of *M. piperita* followed by radiation exposure also resulted in significant decreases in micronucleus frequencies in bone marrow of Swiss albino mice. A significant increase in erythropoietin level was observed at all the studied intervals in leaf extract of *M. piperita* pretreated irradiated animals as compared to control animals (radiation alone). The results of the present investigation suggest the protective effects of leaf extract of *M. piperita* against radiation induced hematopoietic damage in bone marrow may be attributed to the maintenance of EPO level in Swiss albino mice.

INTRODUCTION

Mentha piperita Linn or peppermint (Family – Labiatae) is aromatic and has stimulant and carminative properties. It is being used for allaying nausea, flatulence and vomiting.¹⁾ Leaf extract of *M. piperita* has been shown to have antioxidant and antiperoxidant properties.²⁾ Vokovic-Gacic and Simic³⁾ showed that extracts of mint (*Mentha*) could enhance error-free repair of damage and hence, could be antimutagenic. Samman *et al*⁴⁾ reported that *Mentha piperita* has a chemopreventive effect against shamma-induced carcinogenesis, which could be due to antimutagenic properties. Earlier we have reported that leaf extract of *M. piperita* provide protection against radiation-induced alterations (reduction in villus height, mucosal, total cells and mitotic figures/

crypt section) in intestinal mucosa of mice.⁵⁾ The radioprotective effect of leaf extract of *M. piperita* was also demonstrated by determining dose reduction factor, which was 1.78, by irradiating animals in absence and in presence of leaf extract of *M. piperita* treatment to different doses of radiation (4, 6, 8 and 10 Gy). On the basis of survival percentage the LD_{50/30} values were calculated for control animals and leaf extract of *M. piperita* treated animals were 6.48 Gy and 11.59 Gy, therefore, 8 Gy radiation dose was selected for further investigation.⁶⁾ Also, leaf extract of *M. piperita* administration elevated the counts of endogenous spleen colonies and spleen weight significantly. Pretreatment of leaf extract of *M. piperita* protects the hematological constituents and modulates values of serum acid and alkaline phosphatases activities in Swiss albino mice against gamma irradiation.⁶⁾ Oral administration of leaf extract of *M. piperita* prior to radiation exposure was also found to be effective against chromosomal damage in bone marrow in Swiss albino mice.⁷⁾ Peppermint oil was also been found to protect the hematological constituents in peripheral blood in mice against gamma irradiation.⁸⁾ Recently, we have reported that the chemical composition and chemical constituents

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in plant extracts (*Adhatoda vasica*, *Amaranthus paniculatus*, *Brassica compestris*, *Mentha piperita* and *Spirulina fusiformis*) may have significant role in displaying variation in total antioxidant activity. The differential radioprotective and antioxidant activity of these plant extracts was assigned to different chemical constituents present in each plant extracts.⁹⁾ Present study has been undertaken to evaluate the protective effects of leaf extract of *M. piperita* against radiation induced hematopoietic damage in bone marrow of Swiss albino mice.

MATERIALS AND METHODS

Animals

Adult male Swiss albino mice (*Mus musculus*, 6–8 weeks old, weighing 25 ± 2 g) maintained in the animal house as an inbred colony (procured from Hamdard University, Delhi) were used for the present study. These animals were maintained at a temperature of $24 \pm 3^\circ\text{C}$ and housed in polypropylene cages, as per norms laid down by a Departmental Ethical Committee. After weaning at three weeks of age, the animals were fed standard mouse feed (Hindustan Lever, Delhi, India), and provided tap 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 whole-body exposed to gamma radiation (8 Gy) at the distance (SSD) of 77.5 cm from the source to deliver the dose-rate of 1.59 Gy/min.⁶⁾

Mentha extract (ME)

Mentha piperita Linn. Plant material was collected locally, identified and a specimen was deposited at the Herbarium, Department of Botany, University of Rajasthan, Jaipur (Voucher number-RUBL-19443). Freshly collected leaves were air dried, powdered and extracted with double distilled water (DDW) by refluxing for 36 hr (12 hr \times 3) at 80°C as described previously.⁶⁾

Experimental design

Mice selected from inbred colony were divided into four groups. Animals of Group-I were administered DDW for three consecutive days to serve as normal, while Group-II received leaf extract of *M. piperita* orally (1 g/kg b.wt./day) for three consecutive days. Animals of Group-III received DDW (volume equal to leaf extract of *M. piperita*) to serve as control whereas animals of Group-IV were administered leaf extract of *M. piperita* orally (1 g/kg b.wt./day) for three consecutive days to serve as experimental. After 30 min of treatments on 3rd day, animals of Group-III and IV were exposed to 8.0 Gy gamma radiations. At least six animals

from each group were autopsied at 12, 24, 48 hrs and 5, 10 and 20 days of post - treatments.

Bone marrow smears preparation and cellular counting

Femurs were dissected out from autopsied mice and these were cleaned; their heads were cut and bone marrow was flushed with serum with the help of a syringe. Thin films of the cell suspension were prepared on clean glass slide and stained with Leishman's stain. A total of 500 cells were counted from each slide and the percentage of bone marrow cells was obtained in relation to total cellular counts.

Micronucleus assay

The method of Schmid¹⁰⁾ was employed for the micronucleus assay. The femurs were dissected out and the bone marrow was flushed out, mixed with a vortex mixer, and the cells pelleted by centrifugation. The pellet was resuspended in a few drops of fetal calf serum. Smears were made on pre-cleaned, dry slides, and the slides were air dried and fixed in absolute methanol. The slides then were stained with May-Grün-wald's and Giemsa stains. The micronuclei were scored and reported as micronuclei per 1000 cells.

Erythropoietin (EPO) level

Erythropoietin (EPO) level was measured in serum by SRL Ranbaxy Ltd., Mumbai (India). The test was done with the Immulite Analyzer kit (Catalog No. LKEPZ) manufactured by Diagnostic Products Corporation, USA.

Statistical analysis

The results obtained were expressed as mean \pm SE. Student's 't' test was used to make a statistical comparison between the groups. Significance levels were set at $P < 0.05$, $P < 0.005$ and $P < 0.001$.

RESULTS

The animals treated with leaf extract of *M. piperita* (*Mentha* alone; Group-II) showed no significant change in the number of pronormoblasts and normoblasts, and their values were found near normal values (Table 1). In erythroid series, pronormoblasts and normoblasts showed a gradual decrease, in their number, in control mice (Radiation alone; Group-III), by attaining a minimum value on day 5th (16.8 ± 0.88) and remained significantly low than the normal. When leaf extract of *M. piperita* was given before radiation exposure (Group-IV), the decline in these cells was found to be significantly low as that of the control throughout the experimentation. However, normal count could not be observed even till the end of experiment (*i.e.* 20 days post-irradiation). Megakaryocytes values were decreased gradually as early as 12 hrs interval and remained below normal till 20th day. In experimental animals, decline was comparatively less so counts were higher than control (Table 1). In

Table 1. Per cent variations in Pronormoblasts & Normoblasts and Megakaryocytes in bone marrow after exposure to gamma radiation with or without leaf extract of *M. piperita* treatment in Swiss albino mice

Parameters	Group	Post-treatment Autopsy Intervals					
		12 hrs	24 hrs	48 hrs	5 days	10 days	20 days
Pronormoblasts & Normoblasts	I	28.6 ± 0.22	28.6 ± 0.22	28.6 ± 0.22	28.6 ± 0.22	28.6 ± 0.22	28.6 ± 0.22
	II	28.2 ± 0.48	28.2 ± 0.48	28.2 ± 0.48	28.2 ± 0.48	28.2 ± 0.48	28.2 ± 0.48
	III	22.0 ± 0.54 ^c	21.8 ± 0.60 ^c	20.4 ± 0.52 ^c	16.8 ± 0.88 ^c	17.4 ± 0.28 ^c	16.8 ± 0.48 ^c
	IV	24.6 ± 0.44 ^a	26.8 ± 0.32 ^c	24.6 ± 0.64 ^c	21.8 ± 0.28 ^c	19.4 ± 0.44 ^b	22.6 ± 0.45 ^c
Megakaryocytes	I	3.1 ± 0.62	3.1 ± 0.62	3.1 ± 0.62	3.1 ± 0.62	3.1 ± 0.62	3.1 ± 0.62
	II	2.8 ± 0.50	2.8 ± 0.50	2.8 ± 0.50	2.8 ± 0.50	2.8 ± 0.50	2.8 ± 0.50
	III	3.0 ± 0.34	2.6 ± 0.44	2.8 ± 0.74	2.66 ± 0.44	2.2 ± 0.34	2.0 ± 0.36
	IV	3.1 ± 0.37	2.8 ± 0.74	3.0 ± 0.37	3.1 ± 0.47	2.8 ± 0.34	2.6 ± 0.50

Group I, DDW alone; Group II, *Mentha* extract alone; Group III, DDW + radiation; Group IV, *Mentha* extract + radiation.

Significance levels - ^aP < 0.05; ^bP < 0.005 and ^cP < 0.001, Group II v/s Group I; Group III v/s Group I; Group IV v/s Group III.

Table 2. Per cent variations in myeloid cells of bone marrow after exposure to gamma radiation with or without leaf extract of *M. piperita* treatment in Swiss albino mice

Parameters	Group	Post-treatment Autopsy Intervals					
		12 hrs	24 hrs	48 hrs	5 days	10 days	20 days
Leucoblasts	I	18.2 ± 0.28	18.2 ± 0.28	18.2 ± 0.28	18.2 ± 0.28	18.2 ± 0.28	18.2 ± 0.28
	II	18.4 ± 0.68	18.4 ± 0.68	18.4 ± 0.68	18.4 ± 0.68	18.4 ± 0.68	18.4 ± 0.68
	III	25.4 ± 0.68 ^c	26.0 ± 0.86 ^c	32.8 ± 1.60 ^c	36.8 ± 1.30 ^c	34.2 ± 1.24 ^c	30.6 ± 0.88 ^c
	IV	18.8 ± 0.24 ^c	20.8 ± 0.48 ^c	24.4 ± 0.74 ^b	27.4 ± 0.68 ^c	28.8 ± 0.74 ^a	24.6 ± 0.86 ^b
Myelocytes	I	8.3 ± 0.56	8.3 ± 0.56	8.3 ± 0.56	8.3 ± 0.56	8.3 ± 0.56	8.3 ± 0.56
	II	8.8 ± 0.24	8.8 ± 0.24	8.8 ± 0.24	8.8 ± 0.24	8.8 ± 0.24	8.8 ± 0.24
	III	12.2 ± 0.24 ^c	16.2 ± 0.44 ^c	14.6 ± 0.78 ^c	21.6 ± 1.67 ^c	18.6 ± 1.12 ^c	18.4 ± 0.78 ^c
	IV	10.8 ± 0.20 ^b	10.0 ± 0.47 ^c	12.4 ± 0.60 ^b	16.2 ± 0.60 ^a	14.8 ± 0.74 ^a	11.2 ± 0.87 ^c
Metamyelocytes	I	8.1 ± 0.41	8.1 ± 0.41	8.1 ± 0.41	8.1 ± 0.41	8.1 ± 0.41	8.1 ± 0.41
	II	8.3 ± 0.34	8.3 ± 0.34	8.3 ± 0.34	8.3 ± 0.34	8.3 ± 0.34	8.3 ± 0.34
	III	7.2 ± 0.47	6.4 ± 0.37 ^a	6.2 ± 0.74	5.2 ± 0.54 ^b	4.6 ± 0.47 ^c	4.8 ± 0.68 ^b
	IV	7.8 ± 0.28	7.6 ± 0.64	7.8 ± 0.48	6.8 ± 0.34 ^a	7.2 ± 0.83 ^a	7.0 ± 0.44 ^a
Stab forms	I	13.2 ± 0.73	13.2 ± 0.73	13.2 ± 0.73	13.2 ± 0.73	13.2 ± 0.73	13.2 ± 0.73
	II	12.6 ± 0.78	12.6 ± 0.78	12.6 ± 0.78	12.6 ± 0.78	12.6 ± 0.78	12.6 ± 0.78
	III	10.2 ± 0.34 ^a	9.6 ± 0.72 ^a	8.4 ± 0.26 ^c	6.8 ± 0.24 ^c	7.8 ± 0.58 ^c	8.2 ± 0.86 ^b
	IV	14.6 ± 0.68 ^c	12.2 ± 0.58 ^a	10.8 ± 0.42 ^b	8.4 ± 0.62 ^a	9.2 ± 0.84	10.0 ± 0.58
Polymorphs	I	12.3 ± 0.15	12.3 ± 0.15	12.3 ± 0.15	12.3 ± 0.15	12.3 ± 0.15	12.3 ± 0.15
	II	12.4 ± 0.53	12.4 ± 0.53	12.4 ± 0.53	12.4 ± 0.53	12.4 ± 0.53	12.4 ± 0.53
	III	11.6 ± 0.48	10.4 ± 0.26 ^c	8.8 ± 0.48 ^c	7.4 ± 0.64 ^c	7.9 ± 0.28 ^c	8.8 ± 0.32 ^c
	IV	12.0 ± 0.62	11.2 ± 0.36	9.6 ± 0.28	9.01 ± 0.48	9.6 ± 0.24 ^b	10.6 ± 0.26 ^b
Lymphocytes	I	9.2 ± 0.28	9.2 ± 0.28	9.2 ± 0.28	9.2 ± 0.28	9.2 ± 0.28	9.2 ± 0.28
	II	9.2 ± 0.40	9.2 ± 0.40	9.2 ± 0.40	9.2 ± 0.40	9.2 ± 0.40	9.2 ± 0.40
	III	8.6 ± 0.24	7.4 ± 0.24 ^b	6.8 ± 0.22 ^c	5.8 ± 0.14 ^c	8.2 ± 0.22 ^a	8.4 ± 0.24
	IV	9.0 ± 0.57	8.8 ± 0.48 ^a	8.4 ± 0.60 ^a	7.4 ± 0.48 ^a	9.4 ± 0.64	11.4 ± 0.64

Group I, DDW alone; Group II, *Mentha* extract alone; Group III, DDW + radiation; Group IV, *Mentha* extract + radiation.

Significance levels - ^aP < 0.05; ^bP < 0.005 and ^cP < 0.001, Group II v/s Group I; Group III v/s Group I; Group IV v/s Group III.

myeloid series metamyelocytes, stab/band forms, polymorphs and lymphocytes showed a significant decrease in the control group as compared to normal and this decrease was maximum on day 5 and consequently such counts elevated on 20th day but without regaining the normal number (Table

2). Conversely, the number of leucoblasts and myelocytes showed a significant elevation in control group in comparison to normal, and this increase was maximum on day 5. In leaf extract of *M. piperita* pretreated irradiated animals (Group-IV) this increase was significantly less in compari-

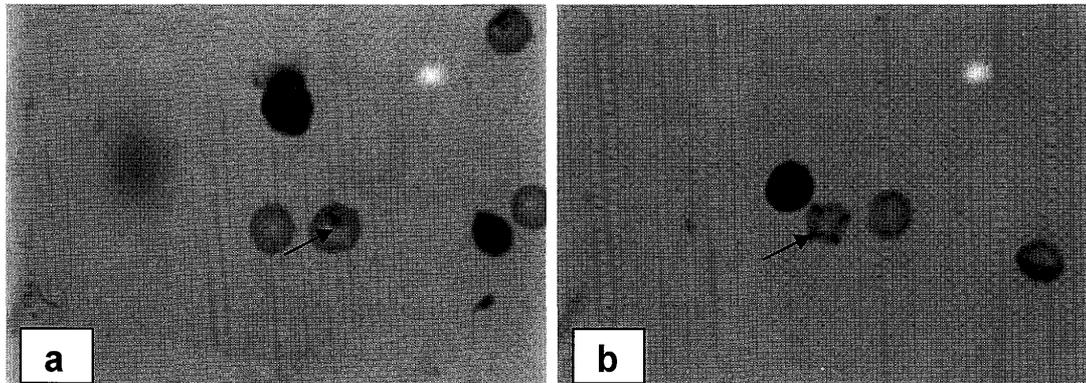


Fig. 1. Radiation-induced micronuclei in bone marrow cells of mice. (a) micronucleated polychromatic erythrocyte (arrow) (b) multiple micronucleated polychromatic erythrocyte (arrow).

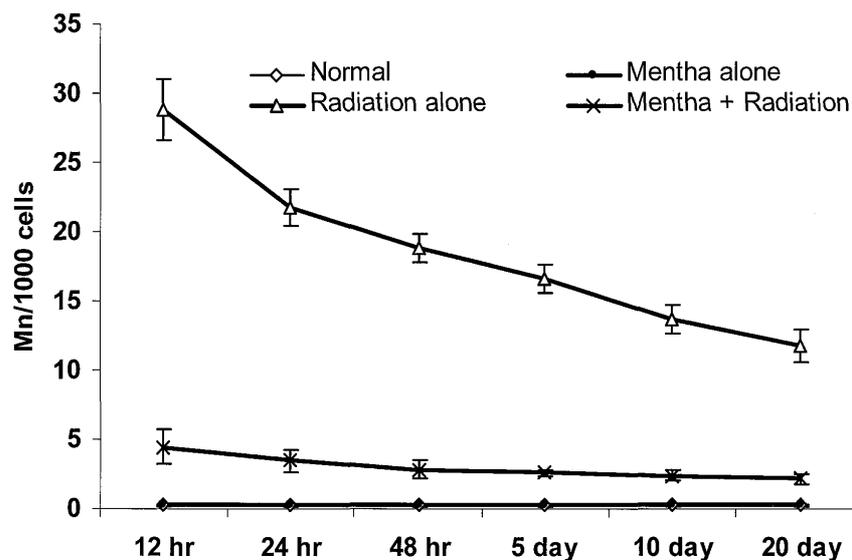


Fig. 2. Effect of Mentha extract on radiation-induced micronucleus frequency in bone marrow of Swiss albino mice.

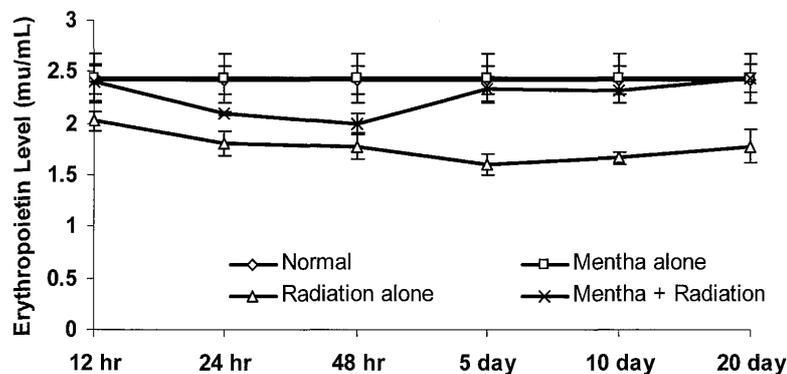


Fig. 3. Variations in erythropoietin level after exposure to gamma radiation with or without Mentha extract treatment in mice.

son to control and normal counts could not be restored even till day 20.

Exposure of Swiss albino mice to gamma radiation resulted in significantly increased frequencies of micronuclei in bone marrow cells. The frequency of micronuclei/1000 cells in the Group III animals was 28.86 ± 2.18 (compared to a frequency of 0.28 ± 0.02 in the Group I; Fig. 1–2). Pretreatment with leaf extract of *M. piperita* followed by radiation exposure (Group IV) resulted in significant decreases in micronucleus frequencies compared to those found in Group III (radiation alone).

In the present investigation, no significant variation in serum EPO was observed in animals treated with leaf extract of *M. piperita* with respect to normal. Exposure to gamma radiation (8.0 Gy) caused a significant decrease in the level of erythropoietin and this decrease was maximum on 5th day (1.60 ± 0.10) in comparison to normal. In animals of leaf extract of *M. piperita* pretreated irradiated (Group-IV), EPO level was higher in comparison to control at all the autopsy intervals (Fig. 3).

DISCUSSION

The results of the present study showed that pretreatment of leaf extract of *M. piperita* protects mice from radiation induced anemia by protecting hematopoietic damage to bone marrow. The protective effect of leaf extract of *M. piperita* was demonstrated by number of pronormoblasts and normoblasts, micronuclei assay in bone marrow and EPO level in peripheral blood of Swiss albino mice. Following lethal exposure, the bone marrow may be so damaged that recovery is impossible.¹¹ It was shown earlier that leaf extract of *M. piperita* 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. Also, leaf extract of *M. piperita* administration elevated the counts of endogenous spleen colonies and spleen weight significantly and protected the hematological constituents in Swiss albino mice against gamma irradiation.⁶ This suggests that leaf extract of *M. piperita* pretreatment provides the protection against hematopoietic damage induced by gamma radiation. Survival of endogenous spleen colony forming cells and granulocyte/macrophage colony forming cells (GM-CFC) by diltiazem was also determined, which indicates recovery from radiation damage in bone marrow.^{12–15}

In the present investigation, pretreatment with leaf extract of *M. piperita* followed by radiation exposure resulted in a significant decrease in micronucleus frequencies compared to those found in Group III (radiation alone). Damage to the chromosomes is manifested as breaks and fragments, which appear as micronuclei in the rapidly proliferating cells.¹⁶ Enhancement in the frequency of micronuclei and chromosomal aberrations has also been reported in the bone marrow

of irradiated mice.^{17,18} Oral administration of leaf extract of *M. piperita* prior to radiation exposure (8 Gy) was found to be effective against chromosomal damage in bone marrow of mice. Irradiated animals exhibited chromosomal aberrations in the form of chromatid breaks, chromosome breaks, centric rings, dicentrics, exchanges and acentric fragments, while animals pretreated with leaf extract of *M. piperita* showed a significantly less number of aberrant cells.⁷

It is generally accepted that erythropoietin (EPO) is produced mainly by the kidneys, released in response to decreased levels of oxygen in the body tissue, and is also produced to a lesser extent by the liver. In the irradiated mice, a pool of erythropoietin sensitive cells has been found to be increased. Erythropoietin not only acts on the existing erythropoietin sensitive cells but also on stem cells that enter in the process of differentiation, enabling these cells to proliferate and differentiate into functioning erythrocytes.¹⁹ CFU-E in the bone marrow is the primary target cell for EPO, and the largest numbers of EPO receptor are formed at the stage of development between the CFU-E and the proerythroblasts.²⁰ Erythropoietin stimulates the CFU-E cells in bone marrow and spleen for the formation of reticulocytes as well as the synthesis of RBC cell membrane proteins and on the set of enucleation.^{21–23} In the present study, an increase in pronormoblasts and normoblasts were observed in leaf extract of *M. piperita* pretreated irradiated animals, which shows that leaf extract of *M. piperita* maintains a high EPO level, that is responsible for an increase in the number of these cells.

Recently, we have reported that the chemical composition and chemical constituents in plant extracts (*Adhatoda vasica*, *Amaranthus paniculatus*, *Brassica campestris*, *Mentha piperita* and *Spirulina fusiformis*) may have significant role in displaying variation in total antioxidant activity. The differential radioprotective and antioxidant activity of these plant extracts was assigned to different chemical constituents present in each plant extracts.⁹ It has been reported that *M. piperita* contains antioxidants like caffeic acid, rosmarinic acid, eugenol and α -tocopherol.^{2,24,25} The radioprotective activity of leaf extract of *M. piperita* observed may be assigned to different chemical constituents present in extract. The possible mechanism of radioprotection by leaf extract of *M. piperita* may be by stimulating/protecting the hematopoietic stem cells against the radiation induced free radical damage by leaf extract of *M. piperita*. The results of the present study suggests the protective effects of leaf extract of *M. piperita* against radiation induced hematopoietic damage in bone marrow may be attributed to the maintenance of EPO level in Swiss albino mice.

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