

Mentha piperita (Linn) leaf extract provides protection against radiation induced alterations in intestinal mucosa of Swiss albino mice

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Received 23 April 2002; revised 13 August 2002

Intestinal protection in mice against radiation injury by *M. piperita* (1 g/kg body weight/day) was studied from day 1 to day 20 after whole body gamma irradiation (8 Gy). Villus height, goblet cells/villus section, total cells, mitotic cells and dead cells/crypt section in the jejunum are good parameters for the assessment of radiation damage. There was significant decrease in the villus height, number of total cells and mitotic cells/crypt section, whereas goblet cells and dead cells showed significant increase after irradiation. *Mentha* pretreatment resulted in a significant increase in villus height, total cells and mitotic cells, whereas goblet cells and dead cells showed a significant decrease from respective irradiated controls at each autopsy day. The results suggest that *Mentha* pretreatment provides protection against radiation induced alterations in intestinal mucosa of Swiss albino mice.

The small intestine represents one of the major dose limiting normal tissues in radiotherapy because of its high radiosensitivity. Reducing the damage to small intestine using chemical protectors, particularly during radiotherapy of the pelvic and abdominal cancers, can increase the patient's tolerance to radiation. It is generally accepted that the cause of death after a dose of radiation less than 5 Gy that results in the death of animals in 10-15 days is because of hematopoietic failure. However, it is an established fact that the gastrointestinal syndrome after total body irradiation also includes hematopoietic components¹⁻³. Thus, in addition to stimulation of hematopoietic cells, other pathways may be required to achieve therapeutic efficacy in radiotherapy. The *Mentha* treatment protects the hematological constituents and serum phosphatases activity in Swiss albino mice against gamma irradiation^{4,5}. Therefore, the present study has been undertaken to determine the radioprotective effect of leaf extract of *Mentha piperita* Linn. on mouse jejunum by using parameters like villus height, goblet cells/villus section, total cells, mitotic cells and dead cells/crypt section as end points.

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.

***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.

Mice from both the groups were autopsied at 1, 2, 5, 10 and 20 days of post-irradiation. A minimum of 5 animals from each group were sacrificed and jejunum was fixed in Bouin's fixative. Sections (5 µm thick)

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were cut and stained with hematoxylin and eosin (H&E). The following parameters were studied to assess the radioprotective effect of *Mentha* extract on intestinal mucosa:

a) villus height; b) number of goblet cells/villus section; c) number of dead cells/crypt section; and d) number of total cells and mitotic cells /crypt section.

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

Results

Control animals (irradiation alone) showed a maximum damage to intestinal mucosa on day 2 post-irradiation followed by recovery at later intervals in the present study, however, normalcy could not be restored even till day 20. In this group, a significant decrease in villus height and number of total cells/crypt section was observed throughout the study. Villus height showed maximum decrease as early as at day 2 ($277.16 \pm 20.80 \mu\text{m}$, $P < 0.001$). However, thereafter a gradual increase was noted but villus height remained below the normal level till day 20 (Table 1). The number of total cells/crypt section was reduced maximally (67.62%) and mitotic cells were completely absent at day 2 in control animals. On day 5 onwards the number of mitotic cells were found to be increased but remained below the normal range even at day 20 i.e. last autopsy day of the study (Figs 1 and 3 a-h). A significant increase in the number of goblet and dead cells was observed at day 1 and 2 respectively in control animals (Fig. 2). Thereafter, the number of these cells started to decline towards

normal range at later intervals. However, the number of dead cells/crypt section was reached to normal on day 20 but the number of goblet cells remained significantly higher than normal even at day 20 (Fig. 2).

In *Mentha* pretreated irradiated animals maximum decrease in villus height was observed again on day 2 ($323.55 \pm 18.72 \mu\text{m}$) but the decrease was significantly less as compared to control group. The number of total cells and mitotic cells/crypt section was found to be significantly higher than control at each autopsy day. The maximum decrease in the number of crypt cells and mitotic cells was observed at day 2 (79.91% and 26.31% respectively). Thereafter, the number of these cells increased and reached to normal level on day 20. The increase in number of goblet cells and dead cells was found to be much less as compared to control in *Mentha* pretreated irradiated animals at each studied autopsy day. However, the number of goblet cells/villus section remained higher than normal at each autopsy day till day 20 but the number of dead cells/crypt section gained normalcy on day 10 and onwards (Figs 2 and 3 a-h).

Discussion

A significant decrease in the height of villus was observed in control animals exposed to 8 Gy gamma radiation. The number of total cells and mitotic cells/crypt section was reduced maximally at day 2.

Table 1—Quantitative changes in villus height in jejunum of mice with or without *Mentha* treatment and exposed to 8 Gy gamma radiation

[Values are mean $\pm$ SE from 5 animals in each observation]

Autopsy time (in days)	Villus height (μm)	
	Control	Experimental
1	291.36 ± 10.87^c	342.18 ± 17.04^a
2	277.81 ± 20.80^c	323.55 ± 18.72
5	355.74 ± 25.46^b	408.25 ± 16.31
10	370.98 ± 13.42^c	430.27 ± 17.66^a
20	377.76 ± 18.33^b	486.17 ± 10.05^c

Normal value: $508.2 \pm 22.85 \mu\text{m}$

Statistical comparison: Control/Normal: Experimental/Control

Control = Control = Irradiation alone (8Gy)

Experimental = *Mentha* + irradiation

P values: ^a < 0.05 , ^b < 0.005 , ^c < 0.001

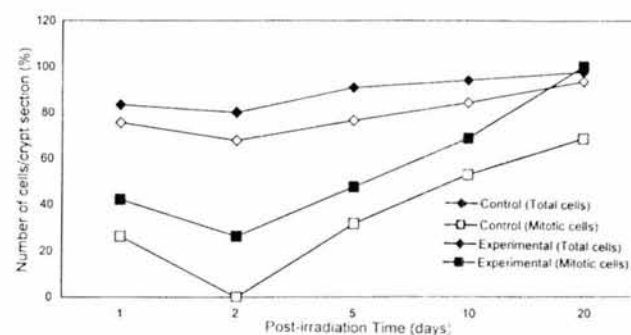


Fig. 1—Total and mitotic cells/crypt section of mice exposed to 8 Gy gamma radiation with or without *Mentha* pretreatment

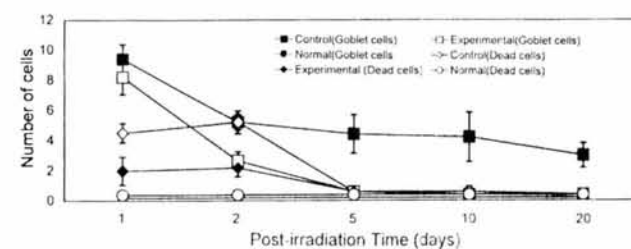


Fig. 2—Number of goblet and dead cells in mice exposed to 8 Gy gamma radiation with or without *Mentha* pretreatment



Fig. 3—Photomicrographs of intestinal crypts of Swiss albino mice exposed to 8 Gy gamma radiation with or without *Mentha* pretreatment [a: normal cell population (X500); b: increased goblet cells in control animal at day 1 (X200); c: dead cells in control animal at day 2 (X500); d: dead cells in experimental animal at day 2 (X500); e: mitotic cells (arrest of mitosis, clumping of chromosomes) in control animal at day 5 (X500); f: mitotic cells (normal) in experimental animal at day 5 (X500); g: normal structure in experimental animal at day 20 (X100); h: normal structure in experimental animal at day 20 (X500)]

However, a significant increase in goblet cells/villus section and dead cells/crypt section was observed at day 1 and 2 respectively, in control animals.

A major part in the radiation damage to the intestinal epithelium is played by interphase damage causing the death of cells immediately after irradiation. The gastrointestinal syndrome includes various mechanisms important for a lethal effect. They include baring of the villi and infection, damage to the blood vessels, and disturbance of the balance of liquids and electrolytes⁶. The crypt is the proliferative unit supplying cells for the maintenance of villus integrity and as such assumes a central role in the intestinal response to radiation exposure⁷. Ionizing radiation affects the cells in all phases of the proliferating cycle but the degree to which these are affected is dependent on the phase in which they were at the time of irradiation⁸. Decline in the percentage of mitotic cells at early intervals may be attributed to a block of cells in G₂ phase of cell cycle and to prolongation of mitotic process^{9,10}. Secondly, dividing cells are highly radiosensitive and their direct killing by radiation exposure may be one of the major factors of reducing the percentage of dividing cells in the present study.

Potten *et al.*¹¹ explained the reduction in the crypt cellularity on the basis that migration of cells from the crypt to the villus continues over the first 24 hr post-irradiation in spite of the fact that cell division is inhibited/or severely reduced. Potten *et al.*¹² further suggested cell death and lack of mitoses as possible reasons for the significant reduction observed in the crypt epithelial cells exposed to 8 Gy radiation. The increase in goblet cell number can be attributed to radiation induced discharge of cells. Radiation exposure slows down the normal differentiation process¹³.

Mentha pretreated irradiated animals showed a significant and consistent increase in villus height and reached to normal by day 20 post-irradiation. The number of total cells and mitotic cells/crypt section was also found to be significantly higher than control at each autopsy day. The number of goblet cells and dead cells was very high in control group as compared to *Mentha* pretreated irradiated animals.

These results are in good agreement with Uma Devi *et al.*¹⁴ who studied the radioprotective effect of 2-mercaptopyrionylglycine on the intestinal crypt of Swiss albino mice after Cobalt-60 irradiation and observed that MPG afforded considerable protection to the intestinal crypts by causing an early recovery from mitotic inhibition followed by an early regeneration of the crypt epithelium.

Evans *et al.*³ suggested that the protection of GI tract by IL1 after total body irradiation was mainly due to the enhanced recovery of bone marrow progenitors in IL1 treated mice. An earlier study by Samarth *et al.*⁴, showed that aqueous extract of *Mentha* protects Swiss albino mice against gamma radiation, and observed significantly higher values of blood corpuscles (RBC/WBC), hematocrit percentage (Hct) and hemoglobin (Hb) level in *Mentha* pretreated irradiated animals as compared to irradiated control animals. Further, a significant enhancement in the number of endogenous spleen colonies has been observed in *Mentha* pretreated irradiated animals⁴. These results suggested that one of the mechanisms of radioprotection offered by *Mentha* extract is due to stimulation/protection of hematopoietic system.

Increase in acid phosphatase activity can be one indicator of cell death. Samarth *et al.*⁵ also reported that *Mentha* extract provides the protection against radiation damage by exhibiting a significant decrease in serum acid phosphatase activity and a remarkable increase in the serum alkaline phosphatase activity in experimental animals as compared to control. Therefore, the decrease in acid phosphatase level could be a consequence of decrease in cell death. Maximum increase in serum acid and alkaline phosphatase activity was recorded at 48 hr after exposure in control animals may reflect the high cell damage and early cell death.

Ionizing radiation is a highly efficient cytotoxic agent. An X-ray dose of 1.5 Gy produces approximately 10⁻⁶ M radicals in cells¹⁵. DNA is the critical target for cell killing by ionizing radiation, and there is growing evidence that the particular lesions that are responsible are DNA double-strand lesions, such as DSB²⁰. While, damage to other biological molecules does occur and is potentially cytotoxic.

Dietary substances are known to contain quite a few micronutrients, essential anti-oxidants, complexing/chelating agents etc. which help in defending from the onslaught of free radicals by their antioxidant properties²⁴, besides detoxifying the toxic materials produced due to metabolic processes and enhancing their clearance from body. *Mentha* extract contains eugenol, caffeic acid, rosmarinic acid and α -tocopherol²¹⁻²³. These compounds have shown to have antioxidant properties and thus inhibit lipid peroxidation. Samman *et al.*²⁵ reported that *Mentha piperita* has a chemopreventive effect against shamma (a complex mixture of powdered tobacco, slaked lime, oils, spices and other additives, which has been linked

to oral cancer in Saudi Arabia) induced carcinogenesis, which could be due to antimutagenic properties. Vokovic-Gacis and Simic²⁶ showed that extracts of mint (*Mentha*) could enhance error-free repair of damage and, hence, could be antimutagenic. A combination of antioxidative processes and antimutagenic activities via modulation of DNA repair processes may be held responsible for the anticarcinogenic²⁶ and radioprotective effects of *Mentha*.

Thus, the results from the present study suggests 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.

Acknowledgement

Senior Research Fellowship (SRF) to RMS from CSIR, New Delhi is thankfully acknowledged. Authors are also thankful to Prof. D. P. Agarwal, Head, Dr. K. S. Jheeta and A. A. Chougule (RSO), Department of Radiotherapy, SMS Medical College and Hospital, Jaipur for radiation facility and dosimetry respectively.

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