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Modulatory effect of *Mentha piperita* (Linn.) on serum phosphatases activity in Swiss albino mice against gamma irradiation

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Mentha extract (ME; 1 g/kg body wt) given orally for three consecutive days prior to whole body irradiation (8 Gy) showed modulation of activity of serum phosphatases in albino mice. Values of acid phosphatase activities were significantly higher in untreated irradiated group throughout the experiment. Irradiated animals pretreated with ME showed significant decline in acid phosphatase activity as compared to untreated irradiated animals at all autopsy intervals and attained normalcy at day 5. A marked decrease in serum alkaline phosphatase activity was recorded in both irradiated groups. However, in ME pretreated irradiated group, values of alkaline phosphatase activity remained significantly higher than untreated irradiated animals at all intervals and attained normalcy from day 5 onwards.

Use of plant preparations as food stuff, flavouring agents, dyes, insecticides as well as CNS active, cardioactive, hypolipidaemic, antimicrobial and antitumor agents are examples of immense chemical diversity in plants. Recently, interest has been generated to search the potential drugs of plant origin that are capable of modifying immune responses[1] (immunomodulators) and radiation responses (radioprotectors/sensitizers) with minimum side effects. Plant extracts like ginseng[2], garlic[3], *spirulina*[4], *ocimum*[5] and herbal drug preparations such as Liv.52 (Ref. 6), rasayanas[7] have been found to have radioprotective effects. Plant products appear to have an advantage over synthetic compounds in terms of low/no toxicity at effective dose.

Peppermint (*Mentha piperita* Linn.) belonging to the family Labiatae is a perennial, glabrous, strongly scented herb which grows to a height of 30-90 cm. The herb is considered aromatic, stimulant and carminative and used for allaying nausea, flatulence and vomiting[8]. *Mentha* extract is known to contain caffeic acid, rosmarinic acid and α-tocopherol,[9-11] whose antioxidant and anti-peroxidant activities have been demonstrated[10]. Aqueous extract of *Mentha* has also been screened for antibacterial activity against *Pseudomonas solanacearum*[12]. In the present study, we have evaluated the modulatory effects of aqueous extract of *Mentha piperita* (Linn.) on serum phosphatases activity against lethal dose of gamma radiation (8 Gy) in Swiss albino mice.

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Animals—Adult male Swiss albino mice (6-8 weeks old; weighing 25±2 g) from an inbred colony (procured from Jamia Hamdard, Delhi) maintained at the animal house of the Institute were used for the present study. The animals were maintained on the standard mice feed and water *ad libitum*. Tetracycline water, once a fortnight, was given as a prevention against infection.

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 and the whole body of these animals was exposed to gamma radiation (8 Gy) at the dose rate of 1.59 Gy/min at a distance (SSD) of 77.5 cm from the source.

Mentha extract (ME)—Fresh leaves of *Mentha piperita* (Linn.) were collected locally. The leaves were air dried, powdered and extracted with double distilled water by refluxing for 36 hr (12 hr×3). The extract thus obtained was vacuum evaporated so as to make it in powder form. The extract was redissolved in double distilled water just before oral administration was also used for oral injections/administration as desired.

Dose selection—Dose selection of *Mentha* extract (ME) was done on the basis of drug tolerance study. Various doses of ME (0.25, 0.50, 1.0, 2.0 and 4.0 g/kg body wt) were tested against gamma irradiation (8 Gy). Optimum dose thus obtained was used for experimentation in detail.

Experimental design—Mice selected from inbred colony were divided into two groups. Animals of one

group were given orally ME (1 g/kg body wt) for three consecutive days to serve as an experimental group while mice of the other group received equal volume of double distilled water (DDW) to serve as control. After 30 min of last treatment, animals of both the groups were exposed to gamma radiation (8 Gy).

The animals were autopsied at 6, 12, 24, 48 hr and 5, 10, 20 and 30 days of post-irradiation. Blood was collected by cardiac puncture and serum was separated. Activity of acid phosphatase and alkaline phosphatase was assayed by using commercially accessible kits (Span Diagnostics Ltd.).

Statistical analysis—The data was subjected to Student's *t* test for comparison between the groups. The values are expressed as mean±SEM.

The animals with ME treatment (alone) did not show any significant change in serum acid phosphatase and alkaline phosphatase activity and values were near normal (Table 1).

In the control group (untreated irradiated) a significant elevation in serum acid phosphatase activity was noticed. A considerable increase was recorded at 12 hr reaching a peak at 48 hr (8.0808±0.12 KAU). The level declined subsequently on day 5 but elevated further and remained higher than normal till their last survival (day 20).

In experimental group (ME pre-treated irradiated) a significant fall in acid phosphatase activity was noticed throughout the experiment as compared to the control (Table 1). The level came down to normal on day 5 with subsequent increase at day 10 and 20 and returned towards normal at last autopsy interval i.e. day 30 (2.7679 ± 0.25).

A remarkable decrease in serum alkaline phosphatase activity was recorded at all intervals. However, maximum decline was noted at 48 hr and day 10 after post-irradiation in untreated irradiated animals. No animal survived till day 30 in this group. In experimental animals (ME pretreated irradiated), the activity of the enzyme exhibited a significant rise above control and attained the normal value at day 5, elevated further at days 10 and 20, and decreased at the end of experimentation (day 30).

Present study revealed an increase in serum acid phosphatase activity after radiation exposure. A similar increase in activity of acid phosphatase after irradiation has also been reported at sublethal doses[13]. Acid phosphatase is localized in cellular lysosomes and change in activity of lysosomal enzymes takes place following whole-body irradiation. An enhanced Golgi activity and peroxidation of lysosomal membranes after irradiation causing lysis of membrane and oozing out of the enzyme are attributed to an increased acid phosphatase level[14]. Release of enzymes from lysosomes may be due to activation of preexisting latent enzymes or due to synthesis of new lysosomes as a consequence of radiation[15].

It is well known that radiations increase the permeability of membranes of several cellular organelles and hence increase in serum acid phosphatase activity was seen after irradiation. In untreated irradiated group rise in acid phosphatase activity till 48 hr can be attributed to the gastrointestinal syndrome, with recovery at day 5. However, further rise can be assigned to other factors like hematopoietic injury. It has been earlier reported (Samarth *et al.*) in our laboratory that aqueous extract of *Mentha* shows radioprotection in Swiss albino mice against lethal dose of gamma irradiation. Dose reduction factor (DRF) value is 1.7. Significant higher values of blood corpuscles (RBC/WBC), Hct percentage and Hb level have been measured in ME pretreated irradiated animals as compared to irradiated (control) animals. Further, a

Table 1—Variation in serum phosphatases activity of mice with or without ME treatment following gamma irradiation (8 Gy) [Values are mean±SE]

Groups	Intervals	Serum phosphatase activity in KAU	
		Acid	Alkaline
C	6 hr	5.7730±0.12***	3.6017±0.14***
Ex		4.7189±0.09***	5.3763±0.11***
C	12 hr	7.4585±0.29***	3.7347±0.11***
Ex		4.1960±0.11***	5.1102±0.09***
C	24 hr	6.9020±0.07***	3.3837±0.18***
Ex		4.0606±0.13***	4.9878±0.22***
C	48 hr	8.0808±0.12***	2.6432±0.09***
Ex		4.4600±0.02***	5.6495±0.19***
C	5 days	4.8806±0.28***	3.9275±0.06***
Ex		2.4023±0.07***	6.7095±0.11***
C	10 days	6.7063±0.14***	2.6436±0.17***
Ex		3.5633±0.05***	7.0199±0.07***
C	20 days	7.3639±0.15***	2.7740±0.17***
Ex		3.0331±0.07***	7.1281±0.12***
C	30 days	N.S.	N.S.
Ex		2.7679±0.25	6.6414±0.05
Normal		2.5911±0.08	6.7107±0.07
ME		2.3737±0.06	6.2675±0.32

C—Untreated irradiated; Normal—No treatment; Ex—ME pretreated irradiated; ME—ME treated unirradiated.
N.S.—Not survived; Significant at ***—$P < 0.001$
Statistical comparison of the groups : Control V/s Normal; Experimental V/s Control

significant enhancement in the number of endogenous spleen colonies has been observed in ME pretreated irradiated group. These observations suggest that one of the mechanisms of radioprotection offered by ME may be due to stimulation/protection of hematopoetic system[22]. In *Mentha* pretreated irradiated group, after day 5, acid phosphatase activity remained higher than normal, but was significantly lower than the respective controls.

In the present investigation, serum alkaline phosphatase activity was found to decline after irradiation at all the intervals studied. This is in agreement of the finding of Jacob and Maini[16], who have also reported deterioration in serum alkaline phosphatase activity in male mice after irradiation with 5 Gy gamma rays. Injury to intestinal mucosa is chiefly responsible for the fall in circulating alkaline phosphatase following irradiation[17]. Whole body irradiation with lethal doses produces a marked fall in alkaline phosphatse activity in rats which is dose dependent[18]. Non-exponential losses of activity in alkaline phosphatse after gamma irradiation has been observed and suggested that radical attacks on phosphatase at centres of secondary importance for the enzymatic activity and there is notable destruction of the component amino acid residue during radiolysis[19].

Alkaline phosphatase plays an important role in maintenance of cell permeability and acts on monophosphoesters. Damage to cell membrane caused by radiation may be the reason for declined activity of serum alkaline phosphatase. In untreated irradiated group, declined alkaline phosphatase level may be attributed to the severe damage to GI tract. Post irradiation reduction in alkaline phosphatase may be due to damage of brush border cells and increased permeability of villi cells[20]. Lesions affecting villi reflect in decreased enzyme activity[21].

In the present investigation, it has been observed that *Mentha* extract provides the protection 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. It has been reported that *Mentha* extract contains eugenol, caffeic acid, rosmarinic acid and α-tocopherol[9-11]. These compounds have shown to have antioxidant properties and inhibition of lipid peroxidation[10].

Thus in the present study, the deleterious lipid peroxides responsible for radiation damage was inhibited by *Mentha* which in turn was reflected by decline in serum acid phosphatase and increase in serum alkaline phosphatase activities as compared to untreated irradiated animals.

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