TREATMENT OF MICE WITH A HERBAL PREPARATION (MENTAT) PROTECTS AGAINST RADIATION-INDUCED MORTALITY

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The effect of various doses (0, 5, 10, 20, 40, 80, 100, 120 and 160 mg/kg b. wt.) of 50% ethanolic extract of mentat (a herbal preparation) was studied on the survival of mice exposed to 10 Gy of γ radiation. Treatment of mice with different doses of mentat consecutively for five days before irradiation delayed the onset of mortality and reduced the symptoms of radiation sickness when compared with the non-drug treated irradiated controls. Most of the doses of mentat provided protection against the gastrointestinal (GI) death, however, the highest protection against GI death was observed for 80 mg/kg mentat. This was also true for bone marrow deaths, where the highest number of survivors were observed at 30 days post-irradiation in this group (i.e. 80 mg/kg) when compared with the other doses of mentat. The evaluation of acute toxicity showed that mentat was non-toxic up to a dose of 1.5 g/kg b. wt., where no drug-induced mortality was observed. The LD_{50} dose of mentat was found to be 1.75 g/kg b. wt. Our study demonstrates that mentat can provide good radioprotection at a dose of 80 mg/kg, which is far below its toxic dose. Copyright © 2003 John Wiley & Sons, Ltd.

Keywords: Mentat; mice; survival; radiation; toxicity; radioprotection.

INTRODUCTION

The search for radioprotectors started with the realization of the need for a safeguard against the military use of atomic weapons. With the recognition that normal tissue protection in radiotherapy is as important as the destruction of the cancer cells, the focus of protection research became more therapy oriented. Since the pioneering work of Patt et al. (1949), that cysteine protected mice and rats against radiation-induced sickness and mortality, several chemical compounds and their analogues have been screened for their radioprotective ability. However, the practical applicability of the majority of these synthetic compounds remained limited, owing to their high toxicity at their optimum protective doses (Sweeney 1979). A turning point came with the observation that s-2-(3-aminopropylamino) ethylphosphorothioic acid (WR-2721) showed substantial and selective protection of normal tissues with little or no protection to the solid tumors (Yuhas and Storer, 1980). Unfortunately, the enthusiasm for clinical use of the WR-[2721] was short-lived when it was realized that like many other synthetic compounds, it was highly toxic at its optimum protective dose and was unable to protect the brain and cells of the spinal chord (Turrissi et al., 1983).

The herbal drugs offer an alternative to the synthetic compounds that have been considered either non-toxic or less toxic than their synthetic counterparts. This has given impetus to screen herbs for their radioprotective ability. The compound formulations are extensively used in the Ayurvedic system of medicine to counteract the toxicity of one herb with the other. The herbal preparation Liv. 52, which has been widely used to treat liver disorders, has been reported to protect mice against radiation-induced sickness, mortality, dermatitis, spleen injury, liver damage, decrease in the peripheral blood cell counts, prenatal development and radiation-induced chromosome damage (Saini et al., 1984a,b; Saini and Saini, 1985; Saini et al., 1985; Jagetia and Ganapathi, 1989; 1991). Certain other herbal preparations like brahmarasayana, narasimharasayana, ashwagandharasayana, and amrithaprasam, a group of herbal preparations used to improve the general health, have also been reported to reduce the radiation-induced lipid peroxidation in the liver, and leucopenia in mice (Kumar et al., 1996). Abana, a composite herbal preparation, clinically used in India as a cardioprotective agent has also been reported to protect the mice bone marrow against the radiation-induced micronuclei formation (Jagetia and Aruna, 1997). The extracts of certain plants like Ocimum sanctum, Panax ginseng and Chlorella vulgaris have been reported to protect mice against the radiation-induced mortality (Jagetia et al., 1986; Zhang et al., 1989; Singh et al., 1995).

Mentat, a marketed herbal drug has been clinically used in India to treat neural disorders. It has been reported to accelerate brain function, improve learning ability, increase memory, improve articulation and reduce behavioral disturbances in mentally retarded children (D’Souza and Chavada, 1991; Koti, 1991; Agarwal et al., 1990). It has also been reported to decrease neurotism index, anxiety (Agarwal et al., 1991), correct speech defects (Mehta, 1991) and control...
anger, hostility, hyperactivity and epileptic fits (Shah, 1992). Mentat administration has also been found to increase metrazole-induced seizures in mice and to decrease restraint-induced gastric ulcers in rats (Dadkar, 1991).

The lesson from the experience with radioprotectors worldwide is that animal studies with death as the end-point are the most confirmatory, because the 30 days time period after lethal whole body irradiation clearly indicates the capacity of the drug, in test to modulate the recovery and regeneration of the gastrointestinal epithelium and the hemopoietic progenitor cells in the bone marrow, the two most radiosensitive organs that are essential for sustaining life. There are no reports regarding the radioprotective activity of mentat. Therefore, the present study was undertaken to evaluate the radioprotective effect of various doses of mentat extract on mice exposed to 10 Gy of supra lethal whole-body γ-radiation.

### MATERIALS AND METHODS

#### Composition of Mentat. The drug mentat is a mixture of Bacopa monnieri, Centella asiatica, Evolvulus alsinooides, Valeriana walluchi, Prunus amygdalus, Acorus calamus, Oroxylum indicum, Mucuna prurients, Ellettaria cardamomum, Foeniculum vulgare, Ipomea digitata, Orchis mascula, Zingiber officinale, Celastrus paniculatus, Tinospora cordifolia, Emblica officinalis, Terminalia arjuna, Withania somnifera, Nardostachys jatamansi, Embelia ribes, Terminalia belerica, Terminalia chebula, Myristica fragrans, Syzygium aromaticum in definite proportions.

#### Preparation of the extract. Extract of mentat was prepared by extracting 100 grams of mentat powder (Himalaya Drug Co., Mumbai, India) in 50% ethanol (1 L) at 50 to 60 °C in a Soxhlet apparatus for 72 h. The cooled liquid extract was concentrated by evaporating its liquid contents, with an approximate yield of 20%.

#### Determination of acute drug toxicity. The acute toxicity of mentat was determined according to Prieur et al. (1973) and Ghosh (1982). Briefly, the animals were allowed to fast by withdrawing food and water for 18 h. The fasted animals were divided into several groups of 10 each. Each group of animals was injected with various doses of 0.5, 1.0, 1.25, 1.5, 1.75, 2.0, 3.0, 4.0 and 6.0 g/kg body weight (b. wt.) of freshly prepared extract of mentat intraperitoneally. Animals were provided with food and water immediately after the drug administration. Mortality of the animals was observed up to 14 days after drug treatment. Acute LD\(50\) of the extract was calculated using a computer program for probit analysis.

#### Effect of mentat on the radiation-induced mortality. The required amount of mentat was dissolved in double distilled water and administered intraperitoneally consecutively for 5 days (Jaghetia and Aruna, 1997). The animals were divided into the following groups:

- **DDW plus irradiation group.** The animals of this group were administered with 0.01 ml/g b. wt. of sterile double distilled water (DDW) intraperitoneally.

- **Mentat plus irradiation group.** The animals of this group were injected intraperitoneally with 5, 10, 20, 40, 80, 100, 120 and 160 mg/kg b. wt. of mentat as described above.

#### Irradiation. One h after administration of DDW or mentat on the 5th day, the prostate and immobilized animals (achieved by inserting cotton plugs in the restrainer) were whole-body exposed to 10 Gy of \(^{60}\)Co gamma radiation (Theraton, Atomic energy Agency, Canada) in a specially designed well-ventilated acrylic box. A batch of ten animals was irradiated each time at a dose rate of 1.99 Gy/min at a source to animal distance (midpoint) of 81.5 cm. The animals were monitored daily for the development of symptoms of radiation sickness and mortality. The statistical significance between the treatments was determined by ‘Z’ test.

### RESULTS

#### Acute Toxicity. The dose of mentat was determined by administering the mice with various doses (0.5, 1.0, 1.25, 1.5, 1.75, 2.0, 3.0, 4.0, 6.0 g/kg b. wt.) of mentat. It was found to be non-toxic up to a dose of 1.5 g/kg, where no drug-induced mortality was observed. A further increase in the drug dose to 1.6 g resulted in 20% mortality. An increase in the drug dose to 1.75 g/kg b. wt. caused a 50% reduction in the survival of mice. 80% of the mice died when the drug dose was increased to 1.8 g/kg b. wt. and no animals survived after the administration of 2.0 g mentat. The LD\(50\) of mentat for acute drug-induced mortality was 1.75 g/kg b. wt. (Table 1).

#### Effect of mentat on the radiation-induced decline in the survival of mice. The signs of radiation sickness were observed in the animals of DDW plus irradiation group within 2–4 days after exposure to 10 Gy of γ-radiation. The main symptoms included reduction in food and water intake, irritability, epilation, weight loss, emaciation, lethargy, diarrhea, and ruffling of hair. Facial edema was also observed in a few animals between one and two weeks after exposure. During the second week after exposure there were a few cases of animals exhibiting paralysis and difficulty in locomotion. The first mortality in this group was observed on day 3 and 75% of the animals died within 10 days after irradiation. All the animals died by day 14 post-irradiation.

Daily administration of different doses of mentat (5, 10, 20, 40, 80, 100, 120 and 160 mg/kg b. wt.) for five consecutive days did not induce mortality and hence were considered safe for administration. Treatment for mice with various doses of mentat delayed the appearance or reduced the symptoms of radiation sickness such as reduction in the food and water intake, irritability, epilation, weight loss, emaciation, lethargy, diarrhea, facial edema etc. The pretreatment of mice with various doses of mentat delayed the onset of radiation-induced mortality depending on the drug dose. This delay was longest for 20 mg/kg mentat, where the first mortality was reported by day 9 post-irradiation (Table 2). The shortest delay in the mortality was observed for 160 mg/kg, where the first mortality occurred on day 4 post-irradiation. The analysis of 10
day survival showed that the lowest mortality (25\%) occurred after the administration of 40 and 80 mg/kg, followed by 10 and 100 mg/kg, where 33.33\% animals died within 10 days (Fig. 1). There was a significant reduction in the radiation-induced mortality for the group administered with 10 to 100 mg/kg of mentat \( (p < 0.05) \).

The analysis of thirty day survival revealed a drug dose dependent increase in the survival of irradiated animals up to a dose of 80 mg/kg in the mentat plus irradiation group, where a highest survival of 50\% was observed (Table 2). A further increase in the drug dose to 100 mg resulted in a 33.33\% reduction in the survival when compared with the 80 mg/kg mentat plus irradiation. Above 100 mg/kg, no survivors could be reported. The animal survival increased significantly for 40 and 80 mg/kg mentat pretreated group when compared with the DDW plus irradiated group \( (p < 0.001) \).

Therefore, the optimum protective dose of mentat has been considered to be 80 mg/kg, which increased the survival of mice by 50\% when compared with the DDW plus irradiation group \( (p < 0.001) \). The lower toxicity of mentat may be owing to the presence of several plants in it that could counteract the toxic implications of other components. The synthetic drug radiation by physical or chemical means. The first report of chemical radioprotection \textit{in vivo} has been published by Patt \textit{et al.} (1949) who reported that cysteine protected mice and rats against the radiation-induced sickness and mortality. Since then several synthetic compounds including thiols have been used for the studies of chemical radioprotection. However, the major drawback of these compounds has been their high toxicity at their optimum protective doses (Sweeny, 1979). Therefore, there is a need to screen alternatives, which are non-toxic at their optimum protective dose. The traditional Indian system of medicine, the Ayurveda, uses extensively the plant or plant derived products for the treatment of various ailments. Most of the drugs used in the Ayurveda are compound formulations, which have been formulated in such a way that their toxic implications are negligible at the administered drug doses. Keeping Ayurveda philosophy in mind, mentat (a herbal preparation), which is commonly used to treat neural disorders, stress related diseases and to improve mental faculties has been selected for the evaluation of its radioprotective ability. Mentat was non-toxic up to a dose of 1.5 g/kg, where no drug-induced mortality was observed, and the LD\textsubscript{50} for the drug induced acute mortality was found to be 1.75 g/kg b. wt. The LD\textsubscript{50} for mentat has been reported to be 2400 mg/kg earlier, where the drug was administered orally (Verma and Kulkarni, 1991). The lower toxicity of mentat may be owing to the presence of several plants in it that could counteract the toxic implications of other components. The synthetic drug

**DISCUSSION**

With the realization of deleterious effects of radiation, attempts have been made to mitigate the effects of radiation by physical or chemical means. The first report of chemical radioprotection \textit{in vivo} has been published by Patt \textit{et al.} (1949) who reported that cysteine protected mice and rats against the radiation-induced sickness and mortality. Since then several synthetic compounds including thiols have been used for the studies of chemical radioprotection. However, the major drawback of these compounds has been their high toxicity at their optimum protective doses (Sweeny, 1979). Therefore, there is a need to screen alternatives, which are non-toxic at their optimum protective dose. The traditional Indian system of medicine, the Ayurveda, uses extensively the plant or plant derived products for the treatment of various ailments. Most of the drugs used in the Ayurveda are compound formulations, which have been formulated in such a way that their toxic implications are negligible at the administered drug doses. Keeping Ayurveda philosophy in mind, mentat (a herbal preparation), which is commonly used to treat neural disorders, stress related diseases and to improve mental faculties has been selected for the evaluation of its radioprotective ability. Mentat was non-toxic up to a dose of 1.5 g/kg, where no drug-induced mortality was observed, and the LD\textsubscript{50} for the drug induced acute mortality was found to be 1.75 g/kg b. wt. The LD\textsubscript{50} for mentat has been reported to be 2400 mg/kg earlier, where the drug was administered orally (Verma and Kulkarni, 1991). The lower toxicity of mentat may be owing to the presence of several plants in it that could counteract the toxic implications of other components. The synthetic drug

### Table 1. Effect of 50\% alcoholic extract of mentat on the acute toxicity in mice

<table>
<thead>
<tr>
<th>Mentat (mg/kg)</th>
<th>Mortality on different days post drug treatment</th>
<th>Survivors (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>100</td>
<td>5/5</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>100</td>
<td>5/5</td>
<td></td>
</tr>
<tr>
<td>1250</td>
<td>100</td>
<td>5/5</td>
<td></td>
</tr>
<tr>
<td>1500</td>
<td>100</td>
<td>10/10</td>
<td></td>
</tr>
<tr>
<td>1600</td>
<td>1</td>
<td>80</td>
<td>8/10</td>
</tr>
<tr>
<td>1750</td>
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<td>5/10</td>
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<td>4</td>
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<td>0/10</td>
</tr>
<tr>
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<td>5</td>
<td>0</td>
<td>0/5</td>
</tr>
<tr>
<td>2500</td>
<td>5</td>
<td>0</td>
<td>0/5</td>
</tr>
</tbody>
</table>

### Table 2. Effect of various doses of 50\% alcoholic extract of mentat on the survival of mice exposed to 10 Gy of \( \gamma \)-irradiation

<table>
<thead>
<tr>
<th>Mentat (mg/kg)</th>
<th>Mortality on different post-irradiation days</th>
<th>No. of Survivors (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>0 (0)</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>0 (0)</td>
<td>12</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>1 (8.33)</td>
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<tr>
<td>20</td>
<td>2</td>
<td>3 (25%)</td>
<td>12</td>
</tr>
<tr>
<td>40</td>
<td>1</td>
<td>5 (41.6%)</td>
<td>12</td>
</tr>
<tr>
<td>80</td>
<td>1</td>
<td>6 (50%)</td>
<td>12</td>
</tr>
<tr>
<td>100</td>
<td>2</td>
<td>3 (25%)</td>
<td>12</td>
</tr>
<tr>
<td>120</td>
<td>3</td>
<td>0 (0)</td>
<td>12</td>
</tr>
<tr>
<td>160</td>
<td>1</td>
<td>0 (0)</td>
<td>12</td>
</tr>
</tbody>
</table>

\( a = p < 0.001, b = p < 0.02. \)
radioprotective substances have been reported to have a cardinal dose beyond and below which protection is not significant (Thomson, 1962; Jagetia et al., 1986). Another herbal preparation, Liv. 52, has been reported to protect mice against radiation-induced sickness, mortality, dermatitis, spleen injury (Saini et al., 1984), liver damage (Saini and Saini, 1985), and prenatal development (Saini et al., 1985). It has also been reported to protect mice bone marrow cells against radiation-induced micronuclei formation and chromosomal aberrations (Jagetia and Ganapathi, 1989; Jagetia and Ganapathi, 1991).

The pattern of survival in the mentat group was similar to that of the irradiated control group except that mortality was delayed. This clearly indicates the effectiveness of mentat in arresting GI death, where the number of survivors for 5, 10, 20, 40, 80 and 100 mg/kg was significantly higher than that of the irradiated control. This reduction in GI death may be due to the protection of intestinal epithelium, which would have allowed proper absorption of the nutrients. Mentat administration has been reported to decrease restraint-induced gastric ulcers in rats (Dadkar, 1991). Pretreatment of mice with another composite herbal drug, Liv. 52, has been reported to protect the intestinal epithelium against radiation-induced damage (Saxena and Goyal, 1998).

The pretreatment of mice with mentat significantly reduced bone marrow deaths in the mentat plus irradiation group, especially for 40 and 80 mg/kg, where a significant elevation in survival has been observed. This increase in 30 day survival may be owing to the protection afforded by mentat to the stem cell compartment, which continued to supply the requisite number of cells in the survivors. A similar effect has been reported for the yeast polysaccharides (Maisin et al., 1986), the extracts of Ocimum sanctum (Jagetia et al., 1986; Ganansoundari et al., 1997), Panax ginseng (Zhang et al., 1989), Spirulina platensis (Qishen et al., 1989), G. thioic acid, synthesized and tested at Walter Reed Army Hospital has been the most promising compound so far tested for protection against radiation and cancer chemotherapeutic drugs, and has been approved by FDA for use against the chemotherapy-induced-toxicity. It has been reported to provide maximum protection at 500 mg/kg while its LD50 dose has been found to be about 710 mg/kg (Yuhas, 1980). The repeated administration of WR-2721 in cancer patients cause systemic toxicity during clinical trials and has been a deterrent against its acceptance in routine cancer therapy. In humans, doses greater than 400 mg/m² have been reported to cause major toxic symptoms like hypotension, emesis, allergic reactions and fever and less serious effects like somnolence, sneezing and hypocalcemia (Turrisi et al., 1983). However, no such symptoms are associated with the administration of mentat as the effective radioprotective dose of 80 mg/kg b. wt. and the cumulative dose of 400 mg/kg b. wt. is far from the LD50 drug dose of 1.75 g/kg b. wt.

Pretreatment of mice with different doses of mentat resulted in a dose dependent reduction in the radiation-induced mortality up to 80 mg/kg and a further increase in the drug dose resulted in a decline in the animal survival when compared with the 80 mg/kg mentat. The

Figure 1. Effect of different doses of mentat on the survival of mice exposed to 10 Gy γ-radiation. Upper diagrams 10 days survival and lower diagram 30 days survival.
Radiation is an another form of stress and *Withania somnifera*, which is one of the components of mentat has been found to ameliorate radiation-induced stress in mice (Kuttan, 1995). In addition to *Withania somnifera*, *Acorus calamus*, *Bacopa monnieri*, *Celastrus paniculatus*, *Centella asiatica*, *Elettaria cardamomum*, *Embodia ribes*, *Emblica officinalis*, *Evolvulus alsinoide*, *Foeniculum vulgare*, *Ipomea digitata*, *Mucuna pruriens*, *Myristica fragrans*, *Nardostachys jatamansi*, *Orchis mascula*, *Oroxyllum indicum*, *Prunus amygdalus*, *Syzzygium aromaticum*, *Terminalia arjuna*, *Terminalia belerica*, *Terminalia chebula*, *Tinospora cordifolia*, and *Valeriana wallichii* the other plants present in the formulation have been documented to relieve stress in different study systems (Nadkarni, 1976; Satyavathi et al., 1987; CHEMEXCIL, 1992; Warrier et al., 1992). *Zingiber officinale*, has been found to reduce malathion-induced stress by increasing the blood glutathione content in the blood and reducing the lipid peroxidation by maintaining the activities of the antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase in rats (Ahmed et al., 2000), which would have also been responsible for the observed protection against the radiation-induced mortality.

The exact mechanism of action of the mentat is not known, however, it may scavenge free radicals produced by radiation and thus reduce the radiation-induced damage to the cellular DNA. This is supported by our observation, where we have found the scavenging of NO (nitric oxide) free radicals by mentat in vitro (unpublished data). The mentat contains plants like *Zingiber officinale*, *Emblica officinalis*, *Withania somnifera* and *Terminalia belerica* which are reported to possess antioxidant and free radical scavenging properties (Jitoe et al., 1992; Naiwu et al., 1992; Jose and Kuttan, 1995).

Alternatively the presence of mentat before irradiation would have enhanced the release of intracellular glutathione resulting in the observed radioprotection.

The various components of mentat like *Embodia ribes*, *Zingiber officinale*, *Syzzygium aromaticum* and *Elettaria cardamum* (Chitra and Shyamaladevi, 1994, Banerjee et al., 1994; Bharali et al., 1998; Ahmed et al., 2000) have been reported to increase GSH levels. The herbal preparation Liv. 52 has been observed to restore the intracellular GSH levels to normal in rats exposed to γ-radiation (Sarkar et al., 1989). Compound/s that have antioxidant effects are known to have an inhibitory action on lipid peroxidation by restoring the GSH levels to normal and mentat may have reduced the radiation-induced lipid peroxidation resulting in the protection against the radiation damage in the present study. Another herbal preparation, Liv. 52 (Ganapathi and Jagetia, 1995), and the plant extract of *Ocimum sanctum* (Uma Devi and Ganasoundari, 1999) have been found to inhibit radiation-induced lipid peroxidation and resulting in the radioprotection by these drug.

**CONCLUSIONS**

From our study it is clear that mentat, a plant based formulation, provided protection against radiation-induced sickness and mortality and the optimum protective single fraction dose of 80 mg/kg and the cumulative dose of 400 mg/kg is far lower than the LD$_{50}$ (1.75 g/kg) dose. The exact mechanism of action of mentat is not known, however, it may scavenge free radicals produced by radiation and thus inhibit radiation-induced damage to the cellular DNA. Alternatively it may increase the level of endogenous glutathione providing protection against radiation-induced damage. We have observed scavenging of NO (nitric oxide) radicals in vitro by mentat (unpublished data) and this testifies to our belief. Since significant protection is obtained at a very low non-toxic dose the extract may have an advantage over the known radioprotectors available so far. Further investigations are being planned to study the mechanism of action of mentat and its clinical applicability for cancer cure.

**Acknowledgements**

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