

Polyherbal Extract of Septilin Protects Mice Against Whole Body Lethal Dose of Gamma Radiation

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The effect of various doses (5, 10, 20, 40, 60, 80, 100, 120, 140 and 160 mg/kg b. wt.) of 50% ethanolic extract of Septilin (a herbal preparation) was studied on the radiation-induced mortality in mice exposed to 10 Gy of γ -irradiation. Treatment of mice with different doses of Septilin, consecutively for 5 days before irradiation, delayed the onset of mortality and reduced the symptoms of radiation sickness when compared with the non-drug treated irradiated controls. All doses of Septilin provided protection against gastrointestinal (GI) deaths (death within 10 days of irradiation). However, the best protection was observed at 100 mg/kg b. wt. of Septilin, as the number of survivors after 30 days post-irradiation was highest (58.33%) in this group when compared with the other doses of Septilin. The number of survivors was 1.75 fold greater for 100 mg/kg Septilin when compared with the 2-mercaptopyrionylglycine (MPG, 33.33%) which was used as a positive control. The LD₅₀ of Septilin was 1250 mg/kg as against the optimum protective dose of 100 mg/kg. Our study demonstrates Septilin as a good radioprotective agent. Copyright © 2004 John Wiley & Sons, Ltd.

Keywords: Septilin; 2-mercaptopyrionylglycine (MPG); mice; survival; radiation; acute toxicity.

INTRODUCTION

Chemical radiation protection has a history of about five decades, when the first report that the natural amino acid cysteine protected mice against the radiation-induced sickness and mortality appeared (Patt *et al.*, 1949). Since then, several compounds with varied chemical structures and pharmacological properties have been screened for their radioprotective ability in mammals but the practical applicability of the majority of these synthetic compounds remained limited, owing to their high toxicity at their optimum protective doses (Sweeney, 1979).

Herbal drugs offer an alternative to the synthetic compounds and have been considered either non-toxic or less toxic and this has given impetus to screen herbal drugs for their radioprotective ability. Ayurveda, the Indian system of medicine, dating back 5000 years, has been an integral part of Indian culture and materia medica. Ayurveda extensively uses plant-derived compound formulations for the treatment of various ailments after a careful study into the type of the disease (Sivarajan and Balachandra, 1996). Studies carried out in the past decade and a half have shown that herbal preparations like Liv. 52, *Brahmarasayana*, *Narasimharasayana*, *Ashwagandharasayana*, *Amrithaprasham*, *Abana* and *Triphala* reduced radiation-induced damage in different study systems (Saini *et al.*, 1984; Kumar *et al.*, 1996; Jagetia *et al.*, 2002, 2003).

Lessons from experience with radioprotectors world wide suggest that animal studies with death of as the

end point are most reliable, because the 30 days time period after lethal whole body irradiation clearly indicates the capacity of the drug to modulate the recovery and regeneration of the gastrointestinal epithelium and the hemopoietic progenitor cells in the bone marrow. These are the two most radiosensitive organs essential for sustenance of the life. Septilin, an Ayurvedic preparation that is extensively used in India for the treatment of several acute/chronic infections (Ross, 1984) was selected for evaluation of its radioprotective activity in mice exposed to 10 Gy of whole body gamma irradiation.

MATERIALS AND METHODS

The animal care and handling was done according with the guidelines set by the World Health Organization, Geneva, Switzerland and the INSA (Indian National Science Academy, New Delhi, India). Eight to ten week old male Swiss albino mice weighing 30 to 36 g were selected from an inbred colony maintained under the controlled conditions of temperature ($23 \pm 2^\circ\text{C}$), humidity ($50 \pm 5\%$) and light (10 and 14 h of light and dark, respectively). The animals had free access to sterile food and water. Four animals were housed in a polypropylene cage containing sterile paddy husk (procured locally) as bedding throughout the experiment.

Composition of Septilin

Septilin is a mixture of the following plant extracts: *Balsamodendron mukul* (resin) Hook. Ex Stocks (162 mg), *Tinospora cordifolia* (whole plant) (Wild.)

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Miers (49 mg), *Rubia cordifolia* Linn. (bark) (32 mg) *Embelica officinales* Gaertn. (fruit) (16 mg), *Moringa pterygosperma* (leaf) Gaertn. also known as *Moringa oliefera* Lam. Syn. M. (16 mg) and *Glycyrrhiza glabra* Linn. (rhizomes) (15 mg).

Preparation of the extract

The extract of Septilin (SPL) was prepared by extracting 100 g of Septilin powder (Himalaya Drug Co., Mumbai, India) in 50% ethanol (1L) at 50 to 60 °C in a Soxhlet apparatus for 72 h. The cooled liquid extract was concentrated by evaporating its liquid contents. An approximate 27% yield of the extract was obtained.

Preparation of the drug solution

2-mercaptopropionylglycine (MPG). The drug 2-mercaptopropionylglycine (MPG) was a kind gift from Santen Pharmaceuticals Limited, Osaka, Japan. Before each experiment, MPG was freshly dissolved in double distilled water (DDW) so as to give a concentration of 2 mg/ml and the pH of the solution was adjusted to 6.4 by the addition of 0.1 N sodium hydroxide before administration. The required amount of Septilin extract was prepared in normal sterile physiological saline (SAL).

Determination of acute drug toxicity

The acute toxicity of SPL extract was determined according to Prieur *et al.* (1973). 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 viz. 250, 500, 750, 1000, 1250, 1500, 1750 and 2000 mg/kg b. wt. of the freshly prepared extract of Septilin intraperitoneally. Animals were provided with food and water immediately after the drug administration. Mortality of the animals was observed up to 14 days post drug treatment. Acute LD₅₀ of the extract was calculated by converting the percent survival into probit values and plotted against the drug dose (Finney, 1971).

Effect of Septilin on the radiation-induced mortality

To evaluate the radioprotective effect of Septilin, the animals were divided into the following groups:

- (1) *SAL + irradiation group* – The animals of this group were administered with 0.01 ml/g body weight of saline (SAL) intraperitoneally;
- (2) *MPG + irradiation group* – The animals of this group were administered with 20 mg/kg b. wt. of MPG intraperitoneally 30 min before irradiation;
- (3) *SPL + irradiation group* – The animals of this group were injected intraperitoneally with various doses viz. 5, 10, 20, 40, 60, 80, 100, 120, 140 and 160 mg/kg b. wt. of the Septilin extract (SPL), consecutively for 5 days (Jagetiya and Aruna, 1997).

Irradiation

One hour after the last administration of SAL or SPL and 30 min after the single administration of MPG, the prostrate and immobilized animals (achieved by inserting cotton plugs in the restrainer) were whole-body exposed to 10 Gy of ⁶⁰Co gamma radiation (Theratron, 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 80 cm. The animals were monitored daily for the development of symptoms of radiation sickness, and mortality. The statistical significance of the treatments was determined by 'Z' test as described by Abramowitz and Stegun (1972) using the following formula:

$$z = \frac{\hat{p}_1 - \hat{p}_2}{\sqrt{\hat{p}(1 - \hat{p})(1/n_1 + 1/n_2)}}$$

where \hat{p} = (number of successes)/total sample size

RESULTS

Acute Toxicity

The administration of different doses of SPL viz. 0.25, 0.50, and 0.75 g/kg b. wt. did not induce drug related mortality during the whole observation period. However, an increase in the drug dose to 1 g/kg b. wt. SPL caused a 20% reduction in the survival of mice. An increase in SPL dose up to 1250 mg/kg resulted in a 50% reduction in the survival of mice and this dose was considered as LD₅₀ dose. A further increase in SPL dose up to 1.5 g caused 100% reduction in animal survival.

Effect of Septilin and MPG on the radiation-induced mortality

The animals of SAL + irradiation group exhibited signs of radiation sickness within 2–4 days after exposure to 10 Gy of γ -radiation. The main symptoms included reduction in the food and water intake, irritability, epilation, weight loss, emaciation, lethargy, diarrhea, and ruffling of hair. A few animals exhibited facial edema between one and two weeks after exposure. During the second week after irradiation a few animals also showed paralysis and difficulty in locomotion. The first mortality in SAL + irradiation group was observed by day 3, and nearly 89% of the animals died by day 10 post-irradiation (gastrointestinal or GI death), while 100% mortality was observed by day 17 post-irradiation (Fig. 1).

Daily administration of the different doses of Septilin for five consecutive days did not cause any drug-induced mortality (data not shown). Treatment of mice with various doses of Septilin before exposure to 10 Gy delayed the appearance and reduced the symptoms of radiation sickness, like reduction in the food and water intake, irritability, epilation, weight loss, emaciation, lethargy, diarrhea, facial edema etc. The administration of MPG, the positive control, delayed the onset of radiation sickness and the first mortality was observed on day 5 (Fig. 1). The pretreatment of mice with

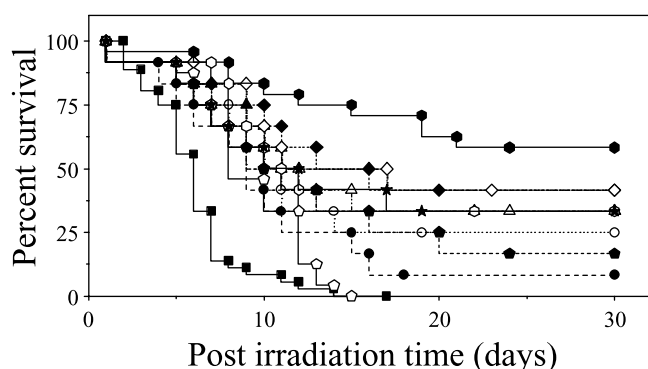


Figure 1. Kaplan Meir's estimate of the survival of mice treated with different doses of SPL and 20 mg/kg MPG before exposure to 10 Gy of γ -irradiation. Closed squares: SAL; closed circles: SPL 5; open circles: SPL 10; closed triangle: SPL 20; open triangle: SPL 40; Closed diamond: SPL 60; open diamond: SPL 80; closed hexagon: SPL 100; open hexagon SPL 120; closed pentagon: SPL 140; open pentagon: SPL 160; closed star: MPG.

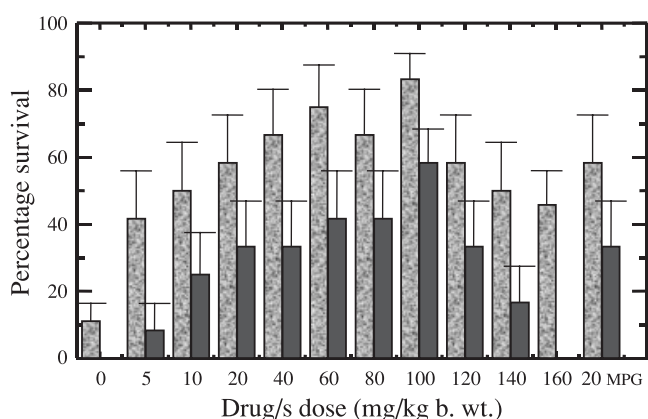


Figure 2. Effect of 5 to 160 mg of septilin extract on the survival of mice exposed to 10 Gy of γ -radiation. Mottled bars: 10 day survival and solid bars 30 day survival with MPG as the positive control. Error bars (95% Confidence limit).

SPL delayed the onset of radiation-induced mortality depending on the drug dose. This delay was longest for 120 mg/kg Septilin, where the first mortality was reported by day 7 post-irradiation, while the shortest delay in the mortality was observed for 5 mg/kg, where the first mortality occurred on day 4 post-irradiation (Fig. 1). The analysis of 10 day survival (GI death) revealed lowest mortality (16.66%) for 100 mg/kg SPL followed by 60 (25%) mg/kg while 40 and 80 mg/kg showed 33.33% mortality, respectively. The MPG pretreatment before exposure to 10 Gy resulted in 41.66% mortality by day 10. Septilin pretreatment increased the survival by 1.43 (100 mg/kg), 1.28 (60 mg/kg) and 1.14 (40 and 80 mg/kg) fold respectively when compared with the MPG (the positive control), which showed only 58.33% survival at its optimum protective dose of 20 mg/kg (Fig. 2).

The analysis of thirty day survival revealed a drug dose dependent increase in the survival of animals up to a dose of 100 mg/kg, in the SPL + irradiation groups, where a highest survival of 58% was observed (Fig. 2). This increase in the survival was 1.25 (60 and 80 mg/kg) and 1.75 fold (100 mg/kg) when compared with the MPG treatment, which showed only 33.33% survival. A further increase in the drug dose to 120 and 140 mg resulted in a 25% and 42% reduction in the survival

when compared with the 100 mg/kg SPL. A still further increase in the drug dose to 160 mg/kg b. wt. resulted in a 100% mortality by day 25 post-irradiation (Fig. 2). Treatment of mice with 80 or 100 mg/kg SPL before 10 Gy irradiation resulted in a significant ($p < 0.001$) elevation in the survival, where the animal survival increased up to 41.66% and 58.33%, respectively when compared with the SAL + irradiation group. As the elevation in the survival was highest for 100 mg/kg SPL (58.33%) this dose was considered as the optimum dose when compared with the other doses of SPL. The optimum radioprotective dose of 100 mg/kg b. wt. of the Septilin was 1/12.5 of the LD₅₀ dose of 1250 mg/kg b. wt.

DISCUSSION

An attempt has been made were to evaluate the radioprotective potential of Septilin, an Ayurvedic herbal preparation used to treat various ailments in India. Irradiation of animals to 10 Gy resulted in radiation sickness within 2–4 days after exposure. The symptoms included reduction in the food and water intake, irritability, epilation, weight loss, emaciation, lethargy, diarrhea, and ruffling of hairs. The death of 89% of the animals exposed to 10 Gy of gamma radiation within 10 days is due to the functional failure of the gastrointestinal (GI) tract (Bond *et al.*, 1965; Uma Devi *et al.*, 1999; Jagetia *et al.*, 2002). The remaining 11% animals died within the next 7 days, exhibiting hemoepoetic syndrome and the characteristic symptoms outlined above. The gastrointestinal epithelium is less sensitive than the bone marrow progenitor cells but as the cell transit time is quick; it is expressed earlier than the hemoepoetic syndrome (Bond *et al.*, 1965). In mice, death within 10 days post-irradiation is due to gastrointestinal damage. Bone marrow stem cells are more sensitive to radiation damage than the intestinal crypt but peripheral blood cells have a longer transit time than the intestinal cells, and hence the gastrointestinal syndrome appears earlier than the bone marrow syndrome. In mice, death due to irradiation from 11 to 30 days, is due to the hemoepoetic damage inflicted by radiation (Bond *et al.*, 1965; Uma Devi *et al.*, 1999; Jagetia *et al.*, 2002).

Pretreatment of mice with different doses of SPL resulted in a dose dependent reduction in the radiation-induced mortality up to 100 mg/kg, and a further increase in the drug dose resulted in a decline in animal survival when compared with 100 mg/kg SPL. A similar observation has been made earlier with other herbal preparations Triphala and Abana (Jagetia *et al.*, 2002, 2003). Earlier studies on radioprotection have shown that an agent in test (for radioprotective action) acts only at a particular dose and above which it may not be protective and can even be toxic (Thomson, 1962).

Pretreatment of mice with SPL provided protection against the radiation sickness and mitigated suffering. As far as the authors are aware, there are no reports regarding the use of SPL as a radioprotective agent and this is the first report to show the radioprotective potential of SPL in mice.

The pattern of survival in the MPG and SPL groups was similar to that of the irradiated control group

except that the mortality was delayed. This clearly indicates the effectiveness of SPL and MPG in arresting GI death, where the number of survivors for all the treatment groups was higher than that of the SAL + irradiation group. The effect of Septilin was superior to that of the MPG, and good radioprotection was observed for 40, 60, 80 and 100 mg/kg SPL treated groups for the GI deaths. The reduction in GI death may also be due to the protection of intestinal epithelium, which would have allowed proper absorption of the nutrients. *Glycyrrhiza glabra*, an important constituent of Septilin, has been reported to mitigate the cysteamine-induced duodenal ulcers by increasing the beta-glucuronidase activity in the Brunner's glands and exhibited anti-ulcerogenic activity against indomethacin-induced gastric ulcers in rats (Nadar and Pillai, 1989).

The treatment of mice with SPL was also much better than MPG in reducing radiation-induced mortality and sickness especially at 100 mg/kg, where a significant elevation in the animal survival has been observed when compared with the MPG pretreatment. This increase in 30 day survival may be owing to the protection afforded by SPL to the stem cell compartment of the bone marrow, which continued to supply the requisite number of cells in the survivors. A similar effect has been reported for the compound formulations like Liv. 52, abana, triphala and the various rasayanas, that have been reported to protect mice against the radiation-induced damage to the hemopoietic system (Kumar *et al.*, 1996; Jagetia *et al.*, 2002, 2003). Septilin has been reported to increase phagocytic activity, leukocytes count, percentage of polymorphs in peripheral blood, proliferation of bone marrow cells and protect against cyclophosphamide-induced myelosuppression and leukopenia in mice (Kumar *et al.*, 1997).

The mechanisms of action of herbal drugs and their extract preparations differ in many respects from that of the synthetic drugs or single substances (Wagner *et al.*, 1988). This effect can be characterized as a polyvalent action and interpreted as additive or in some cases, potentiating. The exact mechanism of action of SPL is not known, however, it may scavenge free radicals produced by radiation and thus reduce radiation-induced damage to the cellular DNA. We have observed

scavenging of NO (nitric oxide) radicals *in vitro* by SPL (unpublished data) and this testifies to our belief that one of the mechanisms of radioprotection by Septilin may be the scavenging of free radicals generated by radiation exposure. Some of the plants used in the Septilin formulation, like *Glycyrrhiza glabra*, *Embelica officinalis* and *Rubia cordifolia*, have been found to possess antioxidant properties (Jose and Kuttan, 1995; Tripathi *et al.*, 1997). *Phyllanthus emblica* has also been reported to possess antioxidant and free radical scavenging activities (Korina and Afanasev, 1997). Alternatively, SPL pretreatment may arrest the radiation-induced decline in the GSH level providing protection against the radiation-induced damage. The extract of *Rubia cordifolia* has been reported to restore glutathione levels after oxidative stress (Pandey *et al.*, 1994).

CONCLUSIONS

From our study it is clear that SPL, a plant based formulation, provided protection against radiation-induced sickness and mortality and that the optimum protective dose of 100 mg/kg is far below the LD₅₀ (1250 mg/kg) dose. Since significant protection is obtained at a low, non-toxic dose, and the effective dose of 100 mg of Septilin is 1/12.5 of the LD₅₀, the extract may have an advantage over known radioprotectors available so far. The exact mechanism of action 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 also increase GSH levels and may reduce the radiation-induced lipid peroxidation.

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