Evaluation of the Radioprotective Action of Geriforte in Mice Exposed to Different Doses of γ-Radiation

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Abstract: The effect of 5, 10, 20, 40 and 80 mg/kg b. wt. of hydroalcoholic extract of geriforte (an Ayurvedic herbal medicine) administered intraperitoneally was studied on the radiation-induced mortality in mice exposed to 10 Gy of γ-radiation. Treatment of mice with different doses of geriforte 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. A maximum protection was observed for 10 mg/kg geriforte, where a highest number of survivors were reported by 30 days post-irradiation and further experiments were carried out using this dose of geriforte. The mice were treated with 10 mg/kg b. wt. geriforte or double distilled water (DDW) and exposed to 7, 8, 9, 10 and 11 Gy of gamma radiation and observed for the induction of symptoms of radiation sickness and mortality up to 30 days post-irradiation. The geriforte treatment protected the mice against the GI death as well as bone marrow deaths and the dose reduction factor (DRF) was found to be 1.14. Toxicity study showed that geriforte was non-toxic up to a dose of 4250 mg/kg, where no drug-induced mortality was observed. The LD50 dose of geriforte was found to be 4750 mg/kg b. wt. To understand the mechanism of action of geriforte, free radical scavenging activity of the drug was evaluated. Geriforte was found to scavenge •OH, O2•−, ABTS•+ and NO• in a dose-dependent manner. Our study demonstrates that geriforte is a good radioprotective agent and the optimum protective dose of 10 mg/kg was 1/475th of the LD50 dose.

Keywords: Geriforte; Mice, Survival; Radiation; Toxicity; Mortality; Free Radical Scavenging.
Introduction

With the realization of deleterious effects of ionizing radiation, a need was felt to protect human beings against these effects by using physical and chemical means. The first report of use of chemicals to protect against the radiation-induced damage appeared in 1949, where Patt et al. (1949) observed that cysteine protected mice and rats against the radiation-induced sickness and mortality. Thereafter, 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 dose (Sweeney, 1979). With the increasing use of radiation in man for medical diagnostic and treatment purposes, it is essential to counteract the deleterious effect of radiation. The herbal drugs have been used by mankind since time immemorial to treat various ailments and offer an alternative to the synthetic compounds, as they are considered either non-toxic or less toxic than their synthetic counterparts. This has given impetus to screen various herbs for their radioprotective ability.

Studies carried out in the past decade and a half have shown that herbal preparations, such as Liv. 52, protected mice against the radiation-induced sickness, mortality, dermatitis, spleen injury, liver damage, decrease in the peripheral blood cell counts, prenatal development, lipid peroxidation and radiation-induced chromosome damage (Saini et al., 1984; Jagetia and Ganapathi, 1989 and 1991, Ganapathi and Jagetia, 1995). The 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 liver, and leucopenia in mice (Kumar et al., 1996). Abana, another herbal preparation, clinically used in India as a cardioprotective agent has also been reported to protect mice bone marrow against the radiation-induced micronuclei formation (Jagetia and Aruna, 1997). Recently, other herbal drugs like triphala, abana, cystone and mentat have also been reported to protect mice against the radiation-induced lethality (Jagetia et al., 2002 and 2003; Jagetia and Baliga, 2002 and 2003).

Geriforte, a polyherbal drug belonging to rasayana group of medicines is being used for the treatment of several disorders in Ayurveda, the Indian system of medicine. In the Ayurveda, the word rasayana, is a term used for a group of herbal preparations that produces sturdiness of the body, sense organs and teeth, prevent wrinkles in skin, graying of hair and promote immune functions and intellect and render longevity to life. Geriforte has been clinically used in India to treat various geriatric and stress-related disorders for more than four decades. This formulation has also been reported to prevent the stress-induced deleterious effects like gastric ulcers, and adrenal hyperplasia in man, reduce exercise-induced fatigue and stress (Singh et al., 1978; Khandeperker and Kulkarni, 1981). Clinical trials have shown that geriforte therapy helps the patients in combating the day-to-day stress without causing any toxic side effects (Shah et al., 1990). A comparative assessment of geriforte with Panax ginseng has shown that the former had better ameliorating effect on anoxia than the latter (Prakash and Singh, 1989). The radiation which induces symptoms akin to stress and the
diverse anti-stress properties attributed to geriforte stimulated us to obtain an insight into the radioprotective properties of 50% ethanolic extract of geriforte in mice exposed to different doses of γ radiation.

Materials and Methods

The animal care and handling were done according to the guidelines set by the World Health Organization, Geneva, Switzerland and the Indian National Science Academy (INSA), 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 ± 2°C), humidity (50 ± 5% ) and light (14 and 10 hours of light and dark, respectively). The animals were provided with the sterile food and water ad libitum. Four animals were housed in a polypropylene cage containing sterile paddy husk (procured locally) as bedding throughout the experiment.

Chemicals

Dimethyl sulphoxide (DMSO), deoxyribose, ethylene diamine trichloroacetic acid (EDTA), ascorbic acid, nitroblue tetrazolium, sodium nitroprusside, Greiss reagent and 2,2-azinobis (3-ethyl benzothiazoline – 6-sulphonic acid) diammonium salt (ABTS) were procured from Sigma Chemical Co. (St. Louis USA). Ferric chloride, sodium bicarbonate, sodium chloride, potassium hydrogen phosphate sodium chloride, disodium hydrogen phosphate, potassium chloride and hydrogen peroxide were supplied by the Ranbaxy Fine chemicals (New Delhi, India).

Composition of Geriforte

Geriforte contains Achillea millefolium (3.2 mg), Adhatoda zeylanica (10 mg), Argyeria nervosa (2.5 mg), Asparagus adscendens (10 mg), Asparagus racemosus (20 mg), Berberis aristata (10 mg), Boerhavia diffusa (10 mg), Caesalpinia digyna (10 mg), Capparis spinosa (13.8 mg), Cassyus occidentalis (5 mg), Celastrus paniculatus (10 mg), Centella asiatica (20 mg), Cichorium intybus (13.8 mg), Crocus sativus (5 mg), Curcuma longa (2.5 mg), Eclipta prostrata (2.5 mg), Elettaria cardamomum (2.5 mg), Embelia ribes (5 mg), Glycyrrhiza glabra (20 mg), Mucuna pruriens (10 mg), Myristica fragrans (2.5 mg), Phoenix dactylifera (10 mg), Piper longum (2.5 mg), Shilajit (20 mg), Solanum nigrum (6.4 mg), Sphaeranthus indicus (5 mg), Syzygium aromaticum (2.5 mg), Tamarix gallica (5 mg), Terminalia arjuna (6.4 mg), Terminalia chebula (15 mg), Tinospora cordifolia (10 mg), Trachypermum ammi (2.5 mg), Tribulus terrestris (5 mg), Vitis vinifera (10 mg) and Withania somnifera (30 mg).
Preparation of the Extract

One hundred grams of geriforte powder (Himalaya Drug Co., Bangalore, India) was extracted in 50% ethanol (1 lt.) at 5°C–60°C in a Soxhlet apparatus for 72 hours. The cooled liquid extract was evaporated to get a semi-solid consistency. An approximate 26% yield was obtained.

Preparation of Drug

The required amount of the extract was weighed and dissolved in double distilled water (DDW).

Determination of Acute Drug Toxicity

The acute toxicity of geriforte extract (GFT) was determined according to Prieur et al. (1973) and Ghosh (1984). Briefly, the animals were allowed to fast by withdrawing the food and water for 18 hours. The fasted animals were divided into several groups and each group of animals was injected with various doses viz. 500, 1000, 2000, 3000, 4000, 4250, 4500, 4750, 5000 and 6000 mg/kg b. wt. of freshly prepared extract of geriforte 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 using a computer program for probit analysis.

Mode of Administration

The animals were administered with 0.01 ml/g b. wt. DDW or GFT intraperitoneally, consecutively for 5 days (Jagetia and Aruna, 1997; Jagetia et al., 2002 and 2003; Jagetia and Baliga, 2002 and 2003) and the animals were divided into two groups as follows.

1) DDW + Irradiation: The animals of this group received DDW as described above before irradiation.

2) GFT + Irradiation: The animals of this group were injected with GFT extract before irradiation.

Irradiation

One hour after the last administration of DDW or GFT on the 5th day, the prostrate and immobilized animals (achieved by inserting cotton plugs in the restrainer) were whole-bodily exposed to 0, 7, 8, 9, 10 and 11 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 1.33 Gy/min at a source to animal distance (midpoint) of 94 cm. The following experiments were conducted.
Selection of Optimum Dose

To select the optimum dose of GFT for radioprotection, the animals were divided into two groups as described above. The animals of GFT + irradiation group were administered intraperitoneally with 5, 10, 20, 40 and 80 mg/kg b. wt. of GFT, and animals of both groups were exposed to 10 Gy of γ-radiation. This allowed the preliminary screening of drug dose and 10 mg/kg b. wt. GFT was found to be the best radioprotective dose and therefore, further experiments were carried out using this dose of GFT.

Radioprotective Effect

To ascertain the radioprotective ability of GFT, the animals were divided into two groups as described above. One group of animals received DDW, while the other group was injected with 10 mg/kg b. wt. of GFT before exposure to 7, 8, 9, 10 and 11 Gy of γ-radiation. The animals of both the experiments were monitored daily for the development of symptoms of radiation sickness, and mortality for 30 days post-irradiation. Percent survival was calculated and plotted against the radiation dose. The dose reduction factor (DRF) was calculated as the ratio of the number of live animals by the method of Miller and Tainter (1944).

\[
\text{DRF} = \frac{\text{LD}_{50/30} \text{ of the GFT + irradiation group}}{\text{LD}_{50/30} \text{ of DDW + irradiation group}}
\]

Free Radical Scavenging In Vitro

Hydroxyl radical scavenging activity

The scavenging of hydroxyl (•OH) free radicals was measured by the method described by Halliwell et al. (1987). Briefly, the reaction mixture contained deoxyribose (2.8 mM), KH₂PO₄–NaOH buffer, pH 7.4 (0.05 M), FeCl₃ (0.1 mM), EDTA (0.1 mM), H₂O₂ (1 mM), ascorbate (0.1 mM) and GFT (10–500 µg/ml) in a final volume of 2 ml. The reaction mixture was incubated for 30 minutes at an ambient temperature followed by the addition of 2 ml of trichloroacetic acid (2.8% W/V) and thiobarbituric acid. The reaction mixture was kept in a boiling water bath for 30 minutes, cooled and the absorbance was read at 532 nm in a UV-VIS double beam spectrophotometer (UV-260, Shimadzu Corp, Tokyo, Japan).

Superoxide anion scavenging activity

The scavenging of superoxide (O₂⁻) anion was measured as described by Hyland et al. (1983). Briefly, the reaction mixture contained various concentrations of GFT (10–500 µg/ml), nitroblue tetrazolium and alkaline DMSO. The blank consisted of pure DMSO instead of alkaline DMSO. The absorbance was read at 560 nm using a UV-Visible double beam spectrophotometer (UV-260, Shimadzu Corp, Tokyo, Japan).
Total antioxidant potential was determined by 2,2-azinobis (3-ethyl benzothiazoline – 6-sulphonic acid diammonium salt) (ABTS) assay described by Miller et al. (1996). This technique measures the relative ability of antioxidant substances to scavenge the ABTS** radical cation generated in the aqueous phase. The reaction mixture contained ABTS (0.00017 M), GFT (10–500 µg/ml), and buffer in a total volume of 3.5 ml. The absorbance was measured at 734 nm UV-Visible double beam spectrophotometer.

Nitric oxide scavenging activity

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction as described previously. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide (Marcocci et al., 1994), which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. Sodium nitroprusside (5 mM) in phosphate-buffered saline was mixed with different concentrations of GFT (20–400 µg/ml) and incubated at 25°C for 150 minutes. The samples from the above were reacted with Greiss reagent (1% sulphanilamide, 2% H3PO4 and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromaphore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamime was read at 546 nm and referred to the absorbance of standard solutions of potassium nitrite treated in the same way with Griess reagent.

Statistical Analysis

The Student’s t-test was used for the free radical scavenging studies, while the “Z” test was used for survival studies (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

The results are expressed as percent survival after exposure to various doses of γ-radiation.

Acute Toxicity

The administration of different doses of geriforte (GFT) viz. 500, 1000, 2000, 3000, 4000 and 4250 mg/kg b. wt. did not induce any mortality during the whole period. However, a further increase in the drug dose to 4500 mg/kg b. wt. caused a 20% reduction in the survival of mice, and when the drug dose was increased to 4750 mg/kg, a 50% reduction in the survival of mice was observed. All the animals died after an injection of 5000 mg/kg b. wt of
RADIOPROTECTIVE EFFECT OF GERIFORTE

GFT. The LD_{50} for the drug induced acute toxicity was 4750 mg/kg b. wt.

Selection of Optimum Dose

The daily administration of different doses of GFT (5, 10, 20, 40 and 80 mg/kg b. wt.) for 5 consecutive days did not induce mortality. Therefore, it was considered safe for administration. The first mortality in the DDW + 10 Gy irradiation group was observed on day 4 where 74.4% of the animals died by day 10 post-irradiation, while only 6.7% animals survived at the end of 30 days post-irradiation (Table 1). Mice treated with DDW + 10 Gy irradiation induced the symptoms of severe radiation sickness like 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 GFT either delayed or reduced the severity of radiation sickness. The onset of mortality was also delayed in the GFT + irradiation group when compared with the DDW + 10 Gy irradiation group. The longest delay was observed for 10 and 20 mg/kg GFT, where the first mortality was reported by day 8 post-irradiation (Table 1) and the shortest delay for 5 mg/kg, where the first mortality occurred on day 5 post-irradiation.

Pretreatment of mice with various doses of GFT also had an ameliorating effect on the gastrointestinal tract as evidenced by an increase in the 10-day survival of mice. Majority of the animals (74.4%) of DDW + 10 Gy irradiation group died within 10 days after irradiation, while the GFT pretreatment resulted in an increase in the 10-day survival (Fig. 1). A lowest mortality was observed in the animals pretreated with 10 mg/kg before irradiation and this decline in mortality was significant for 10 mg/kg. Other doses of GFT also reduced the mortality, in comparison with DDW + 10 Gy irradiation. However, a significant elevation in the 10-day survival was observed only for 10 and 20 mg/kg GFT (p < 0.001) treatment (Fig. 1).

Analysis of the 30-day survival revealed a drug dose-dependent increase in the survival of irradiated animals up to a dose of 10 mg/kg in GFT + irradiation group, where a highest survival of 41.7% was observed when compared with the DDW + irradiation group, where 6.7% survivors were observed (Fig. 1). Increase in the drug dose to 20 and 40 mg resulted in 8.3% and 25% reduction, respectively in the survival when compared with the 10 mg/kg GFT + irradiation (Fig. 1). A significant increase in the survival of irradiated animals was observed only for the 10 (p < 0.002) and 20 (p < 0.01) mg/kg GFT + irradiation group, while the highest survival was observed for the former (10 mg/kg). Therefore, the optimum protective dose of GFT was considered to be 10 mg/kg and further experiments were carried out using this dose of GFT.

Radioprotective Effect

The radioprotective effect of GFT was studied in mice pretreated with 10 mg/kg GFT consecutively 5 days before exposure to 7, 8, 9, 10 and 11 Gy of γ-radiation. The animals of DDW + irradiation group exhibited signs of radiation sickness within 2–4 days after exposure.
Table 1. Effect of Various Doses of Geriforte Extract on the Survival of Mice Exposed to 10 Gy of γ-Radiation

<table>
<thead>
<tr>
<th>GFT (mg/kg)</th>
<th>Survivors (Percent)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2 (6.66)</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>2 (16.66)</td>
<td>12</td>
</tr>
<tr>
<td>10</td>
<td>10 (41.66)*</td>
<td>24</td>
</tr>
<tr>
<td>20</td>
<td>8 (33.33)</td>
<td>24</td>
</tr>
<tr>
<td>40</td>
<td>2 (16.66)</td>
<td>12</td>
</tr>
<tr>
<td>80</td>
<td>0 (0.00)</td>
<td>12</td>
</tr>
<tr>
<td>GFT 80 + 0 Gy</td>
<td>12 (100)</td>
<td>12</td>
</tr>
</tbody>
</table>

p < *0.002; †0.01.

Table 2. Effect of 10 mg/kg b. wt. of Geriforte Extract on the Survival of Mice Exposed Different Doses of γ-Radiation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (Gy)</th>
<th>Survivors (Percent)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDW + IR</td>
<td>7</td>
<td>17 (70.83)</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>10 (41.67)</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>2 (8.33)</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0 (0.00)</td>
<td>24</td>
</tr>
<tr>
<td>GFT + IR</td>
<td>7</td>
<td>20 (83.33)</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>16 (66.66)</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>10 (41.66)*</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2 (8.33)</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IR: Irradiation; GFT: geriforte extract. 
P < *0.002.
to different doses of $\gamma$-radiation depending on the irradiation dose. The exposure of mice to higher radiation doses resulted in the early appearance of the symptoms of radiation sickness. The main symptoms included reduction in the food and water intake, irritability, watering of eyes, lethargy, ruffling of hair, diarrhea, weight loss, emaciation and epilation. Facial edema was also observed in a few animals between 1 and 2 weeks after exposure to 9, 10 and 11 Gy. A few animals also exhibited paralysis and difficulty in locomotion during the 2nd week after exposure. The severity of the symptoms increased with the increase in radiation dose.

The whole body irradiation of mice to 7 Gy did not induce mortality in both the groups (Table 2). However, with the increasing dose of radiation, the survival declined in a dose-dependent manner till a nadir in the survival was reached at 11 Gy, where no survivors were reported beyond 14 days post-irradiation in the DDW + irradiation group. With the increase in the exposure dose the onset of mortality was also advanced (Table 2). The survival was plotted on the log, while the exposure dose on the linear scale and the LD$_{50/30}$ was found to be 8.6 Gy for this group.

The pretreatment of mice with 10 mg/kg GFT delayed or reduced the severity of radiation sickness and the onset of radiation-induced mortality when compared with the concurrent DDW + irradiation group (Table 2). This delay in the onset of mortality was by almost 3–4 days in the GFT + irradiation group when compared with the DDW + irradiation group.
The GI deaths were fewer compared to DDW + irradiation group for all exposure doses (Fig. 2a). Similarly, GFT treatment also increased the number of survivors at 30 days post-irradiation (p < 0.002 for 10 Gy), when compared with the concurrent DDW + irradiation group (Fig. 2b). The LD$_{50/30}$ was found to be 9.8 Gy for the GFT + irradiation group, resulting in an increase of 1.2 Gy. The dose reduction factor (DRF) was found to be 1.14 (Fig. 2b).

**Free Radical Scavenging**

The data are shown as percent scavenging of free radical generation in Fig. 3. The GFT inhibited the generation of $\cdot$OH and $O_2^{•−}$ radicals in a dose-dependent manner and a maximum scavenging was observed at 500 µg/ml (Figs. 3a and b). The total antioxidant activity was measured using ABTS assay and the inhibition of ABTS$^{•−}$ radicals showed a dose-dependent scavenging and a peak was observed at 500 µg/ml (Fig. 3c). Similarly, the GFT also showed a dose-dependent scavenging of nitric oxide up to 200 µg/ml. Thereafter, the nitric oxide scavenging declined with increase in the drug concentration and was significant in all assays (Fig. 2d).

**Discussion**

The traditional Indian system of medicine, Ayurveda (Ayu = life, Veda = knowledge), extensively uses the plant-derived compound formulations for the treatment of various ailments after a careful study into the type of the disease. The treatment philosophy of Ayurveda is based on the principle of balance and counter-balance. Plants are complex mixtures of compounds and no single compound can provide the desired activity. Some compounds potentiate a desired therapeutic action, while others reinforce the same, and yet others interact to neutralize and counteract any possible side effects that may exist. Therefore, several plants with the common desired activities and varied undesirable activities are selected so that the final formulation will have a concentrated desired activity and the undesired activities will be diluted or absent altogether (Sharma and Dash, 1998). The effect of compound herbal drug geriforte was evaluated for its radioprotective activity in mice whole body exposed to various doses of $\gamma$-radiation.

The acute toxicity studies revealed the non-toxic nature of geriforte, where the LD$_{50}$ was found to be 4.75 g. A similar effect which had been observed earlier where the toxic dose was found to be between 5–6 g after oral administration (Singh et al., 1980). The lower toxicity of geriforte may lie in its compound formulation, where the presence of several plants in it, could counteract the toxic implications of other components, which is in conformation with Ayurvedic philosophy. The chronic administration of geriforte has been reported to be devoid of mutagenic, teratogenic and carcinogenic activities (Kothari, 1976).

Exposure of mice to ionizing radiation induced the symptoms of radiation sickness and mortality. A similar effect has been previously observed (Bond et al., 1965; Jagetia et al., 2002 and 2003; Jagetia and Baliga, 2002 and 2003). The ionizing radiation at cellular level can induce damage in the biologically important macromolecules such as DNA, proteins,
Figure 2. Effect of 10 mg/kg geriforte extract on the survival of mice exposed to different doses of γ-radiation: DDW + irradiation (squares) and GFT + irradiation (circles). (a) 10-day survival and (b) 30-day survival.
Figure 3. Effect of various concentrations of GFT on the scavenging of various free radicals and ABTS** cation radical. (a) Hydroxyl radical, (b) superoxide anion, (c) ABTS** cation radical, and (d) nitric oxide**.
lipids and carbohydrates in various organs. While some damage is expressed early, the others may be expressed over a period of time depending upon the cell kinetics and the radiation tolerance of the tissues. The proliferating cells are highly sensitive to the effect of radiation; therefore, the effect of whole body irradiation is mainly felt by the highly proliferating germinal epithelium, gastrointestinal epithelium and the bone marrow progenitor cells. Of these, the germinal epithelium does not have any active role to play in the life supporting function of the exposed individual, while the gastrointestinal epithelium and the bone marrow progenitor cells are crucial for the sustenance of life and any damage to these cells will impair the normal physiological processes drastically causing death depending upon the severity of damage. 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 hemopoietic syndrome (Bond et al., 1965). In mice, death within 10 days post-irradiation is due to gastrointestinal damage (Bond et al., 1965; Jagetia et al., 2002 and 2003; Jagetia and Baliga, 2002 and 2003). The bone marrow stem cells are more sensitive to radiation damage than the intestinal crypt; however, the peripheral blood cells have a longer transit time than the intestinal cells and hence the hemopoietic syndrome appears later than the gastrointestinal syndrome. In mice, death due to irradiation from 11 to 30 days is due to the hemopoietic damage inflicted by radiation to the hemopoietic organs like the bone marrow (Bond et al., 1965; Jagetia et al., 2002 and 2003; Jagetia and Baliga, 2002 and 2003).

Pretreatment of mice with different doses of GFT resulted in a dose-dependent reduction in the radiation-induced mortality up to 10 mg/kg and a further increase in the drug dose resulted in a decline in animal survival when compared with the 10 mg/kg GFT. A similar effect has been observed earlier (Jagetia et al., 2002 and 2003; Jagetia and Baliga, 2002 and 2003). The 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). The reason may be that after a particular concentration, a compound may start manifesting its toxic effects. A similar action cannot be ruled out for GFT that has offered an optimum protection at 10 mg/kg, while the higher doses resulted in the decline in protective action of GFT. The pretreatment of mice with GFT provided protection against radiation sickness and mortality.

The LD$_{50/30}$ was found to be 9.8 Gy for the GFT + irradiation group, resulting in an increase of 1.2 Gy. The DRF was found to be 1.14. As far as the authors are aware, there are no reports regarding the use of GFT as a radioprotective agent, and it is probably the first report to show the radioprotective potential of GFT in mice. However, other polyherbal drugs like triphala, cystone, mentat and abana have been reported to protect mice against the radiation-induced sickness and mortality (Jagetia et al., 2002 and 2003; Jagetia and Baliga, 2002 and 2003). Certain other polyherbal preparations like Liv. 52 and abana have been reported to protect mice against the radiation-induced sickness, mortality, dermatitis, spleen injury and radiation-induced chromosome damage (Saini et al., 1984; Jagetia and Ganapathi, 1989 and 1990). The brahmarasayana, narasimharasayana, aswagandha-rasayana and amrithaprasham, a group of herbal preparations used to improve the general health and debility, have also been reported to reduce the radiation-induced lipid peroxidation in the liver and leucopenia in mice (Kumar et al., 1996).
The pattern of survival in the GFT group was similar to that of the irradiated control group except that the mortality was delayed. This clearly indicates the effectiveness of GFT in arresting GI death, where the number of survivors for all the treatment groups was higher than that of the DDW + irradiation group. The administration of different doses of GFT, especially 10, 20 and 40 mg/kg, resulted in a dose-dependent inhibition in the GI deaths. This reduction in GI death may be due to the protection of intestinal epithelium, which would have allowed proper absorption of the nutrients. It has been reported that *Terminalia chebula* and *Glycyrrhiza glabra*, the important constituents of GFT, reduced the cysteamine-induced duodenal ulcers in rats by increasing the β-glucuronidase activity in the Brunner’s glands (Nadar and Pillai, 1989). Further, plants like *Terminalia chebula* and *Glycyrrhiza glabra* have been reported to protect the epithelial cells against the cytopathic effects of influenza A virus and indomethacin-induced gastric ulcers in rats (Badmaev and Nowakowski, 2000; Khayyal *et al.*, 2001). The antimicrobial and antiviral activity of GFT and its constituents plants may have played some role in protecting mice against the radiation-induced GI deaths (Phadke and Kulkarni, 1989; Ahmad *et al.*, 1998).

The treatment of mice with GFT significantly reduced the bone marrow deaths in the GFT + irradiation group, especially for 10 mg/kg, where a significant elevation in the animal survival has been observed. This increase in 30-day survival may be owing to the protection afforded by GFT to the stem cell compartment of the bone marrow, which continued to supply the requisite number of cells in the survivors. The geriforte administration has been reported to reduce the urethane-induced leucopenia in mice (Singh *et al.*, 1980), and it has also been reported to be immunomodulator (Kumar *et al.*, 1982; Rege *et al.*, 1999; Chintalwar *et al.*, 1999). A similar effect has been reported for the compound formulations like Liv. 52, abana, triphala, cystene and the various rasayanas, that have been reported to protect the mice against the radiation-induced damage to the hemopoietic system (Jagetia and Ganapathi, 1989 and 1991; Jagetia and Aruna, 1997; Jagetia *et al.*, 2002 and 2003; Jagetia and Baliga, 2002 and 2003).

The geriforte has also been reported to reduce stress and fatigue in rats and humans (Khandeparkar and Kulkarni, 1981). Since many symptoms of radiation-induced sickness take forms of stress, the radioprotection observed in the present study by GFT may also be due to its stress-relieving property. Geriforte has also been reported to restore and revitalize the reproductive capacity of rats (Kothari *et al.*, 1985). A similar effect of GFT in the other cell renewal systems like bone marrow and the GI tract cannot be ruled out in the present study.

The exact mechanism of action of GFT is not known; however, it may scavenge free radicals produced by radiation and thus reduce the radiation-induced damage to cellular DNA. This contention is supported by the experiments on free radical scavenging, where GFT has been found to scavenge *OH* and *O_2*-*, ABTS** and NO* radicals in a dose-dependent manner, except NO, where the greatest scavenging effect was observed for 200 µg/ml and declined thereafter. Alternatively, GFT pretreatment may arrest the radiation-induced decline in the GSH level providing protection against the radiation-induced damage. The extract of some of the plants that are part of GFT formulation like *Piper longum*, *Ellettaria cardamomum*, *Syzygium aromaticum*, *Curcuma longa* and *Rubia cordifolia* have been
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reported to restore the glutathione level to normal in the liver cells exposed to oxidative stress (Pandey et al., 1994; Bharali et al., 1998), while the others, like Glycyrrhiza glabra, Embelica officinalis, Rubia cordifolia, Vitis vinifera, Terminalia chebula, Boerrhaavia diffusa, Phyllanthus emblica, Curcuma longa, Asparagus racemosus and Eclipta alba have been reported to possess antioxidant properties (Jose and Kuttan, 1995).

The present results suggest that the free radical scavenging and antioxidant property of GFT is most likely to be the mechanism of radiation protection, although other mechanisms like the up-regulation of detoxifying enzymes, metal chelation etc. cannot be ruled out. As the protective dose is extremely low, the observed radioprotective activity may be due to the beneficial activities of other active principles, which may have complimented the radioprotective action of the GFT. If so, the basic principle of Ayurveda, that one ingredient may be principally responsible for an action but other secondary components may be just as important activators or potentiator of this action needs to be remembered (Sharma and Dash, 1998).

Acknowledgments

The authors are grateful to Himalaya Drug Co., Bangalore, India for providing the geriforte powder as a free gift to carry out the study. We also thank Prof. M. S. Vidyasagar, and Dr. J. Velumurugan, Department of Radiotherapy and Oncology, Kasturba Medical College, Manipal, India for providing the necessary irradiation facilities and help in radiation dosimetry, respectively.

References


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