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Evaluation of the radioprotective effect of *Ageratum conyzoides* Linn. extract in mice exposed to different doses of gamma radiation

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Abstract

The effect of various doses (0, 25, 50, 75, 100, 125, 150, 300, 600 and 900 mg kg^{-1}) of the alcoholic extract of the plant *Ageratum conyzoides* Linn. (ACE), on the alteration of radiation-induced mortality in mice exposed to 10 Gy of gamma radiation was studied. The acute toxicity studies showed that the drug was non-toxic up to a dose of 3000 mg kg^{-1}, the highest dose that could be tested for acute toxicity. Administration of ACE resulted in a dose-dependent decline in radiation-induced mortality up to a dose of 75 mg kg^{-1}, the dose at which the highest number of survivors (70.83%) was observed. Thereafter, the number of survivors declined with increasing doses of ACE and a nadir was reached at 900 mg kg^{-1} ACE. Since the number of survivors was highest for 75 mg kg^{-1} ACE, this was considered the optimum dose for radioprotection and used in further studies in which mice were treated with 75 mg kg^{-1} ACE before exposure to 6, 7, 8, 9, 10 and 11 Gy of gamma radiation. The treatment of mice with 75 mg kg^{-1} ACE reduced the severity of symptoms of radiation sickness and mortality at all exposure doses, and a significant increase in survival was observed compared with the non-treated irradiated group. The ACE treatment effectively protected mice against the gastrointestinal as well as bone marrow related death, as revealed by the increased number of survivors at all irradiation doses. The dose reduction factor was found to be 1.3. To understand the mechanism of action, various doses of ACE were evaluated for their in-vitro scavenging action on 1,1-diphenyl-2-picrylhydrazyl (DPPH), a chemically stable free radical. ACE was found to scavenge DPPH radicals in a concentration-dependent manner, indicating that the radioprotection afforded by ACE may be in part due to the scavenging of reactive oxygen species induced by ionizing radiation.

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Introduction

The use of ionizing radiation has become an integral part of modern medicine. It is used for diagnostic as well as therapeutic purposes. In some cases, radiation may be the single best treatment of cancer. However, for many solid tumours, a cure with radiation remains elusive. The radiation therapy of cancer depends on achieving a therapeutic differential between the cancer cell cytotoxicity and normal tissue toxicity. The therapeutic differential may be achieved with chemical radiation sensitizers or protectors. The development of radiation protectors is important not only to enhance the effectiveness of cancer treatment, but also for the study of the underlying mechanisms of radiation cytotoxicity. Some radioprotectors are known to offer protection through a direct effect on the cellular targets of radiation, while others enhance the recovery of normal tissues (Hahn et al 1994).

In the search for natural radioprotectors, investigators have recently focussed their attention on the plant/natural products. The extracts of *Ocimum sanctum*, *Panax ginseng* and *Mentha arvensis* have been reported to protect mice against radiation-induced mortality (Jagetia et al 1986; Zhang et al 1987; Jagetia & Baliga 2002). Certain

herbal preparations such as Liv. 52, abana and triphala have also been reported to protect mice against radiation-induced sickness, mortality, dermatitis, spleen injury, liver damage, decrease in peripheral blood cell counts and radiation-induced chromosome damage (Saini et al 1984, 1985; Jagetia & Ganapathi 1989; Jagetia et al 1993, 2003; Ganasoundari et al 1997a).

Ageratum is derived from the Greek "*a geras*" meaning non-aging, referring to the longevity of the flowers or the whole plant. The specific epithet "*conyzoides*" is derived from "*kónyz*" the Greek name of *Inula helenium*, which it resembles (Kissman & Groth 1993). *Ageratum conyzoides* belongs to the family Asteraceae (Eupatoriae) and is commonly known as Billy Goat Weed. It has been used in various parts of Africa, Asia and South America for curing various diseases. The plant has been used in folk remedies as a purgative, febrifuge and also for ophthalmia, colic, the treatment of ulcers, and as a wound dressing. *A. conyzoides* has been reported to possess diverse medicinal properties (Githens 1948). In some African countries, it has been indicated in the treatment of mental and infectious diseases, as well as headaches and dyspnea (Yamamoto et al 1991). In Brazilian folk medicine, the medicinal teas of *A. conyzoides* are used as anti-inflammatory, analgesic and antidiarrhoeal remedies (Ekundayo et al 1988). Aqueous extracts of leaves or whole plants have been used to treat colic, colds and fevers, diarrhoea, rheumatism, spasms, or as a tonic (Penna 1921; Negrelle et al 1988). A large number of constituents have been identified from the gas chromatography-mass spectrometry analysis of the essential oil of *A. conyzoides*. The plant has been found to possess sabinene, β-pinene, β-phellandrene, limonene and α-terpineol. About 5.3% ocimene was found in the oil collected from the Indian plant, along with sesquiterpenes such as β-caryophyllene, δ-cadinene (Oliveira et al 1993), sesquiphellendrene and caryophyllene epoxide (Katsuri & Manithomes 1967).

A. conyzoides is very rich in polyoxygenated flavonoids, which include 14-polymethoxylated flavones, of which the major compounds include 5′-methoxynobiletin, linderoflavone B and 5,6,7,3′,4′,5′-hexamethoxyflavone (Katsuri & Manithomes 1967; Quijano 1980; Vyas & Mulchandani 1988; Gonzalez et al 1991; Pari et al 1998). These flavonoids have been reported to increase antioxidant enzymes in-vivo (Kong 2000). Similarly, eugenol, which is present in ACE, has been reported to be a good antioxidant (Vidya & Devraj 1999). Lessons from the experience with radioprotectors worldwide indicate that animal studies, with death of the animal as the end-point, are the most confirmatory, because the 30-day time period after lethal whole-body irradiation clearly indicates the capacity of the test drug to modulate the recovery and regeneration of the gastrointestinal epithelium and the haemopoietic progenitor cells in the bone marrow, the two most radiosensitive organs that are essential for life. Its common usage, wide acceptability, diverse medicinal and antioxidative properties stimulated us to study the radioprotective effect of *A. conyzoides* in mice exposed to various doses of whole-body gamma radiation, by evaluating survival for 30 days after irradiation.

Materials and Methods

Experimental design

The animal care and handling was carried out according to the guidelines issued by the World Health Organization, Geneva, Switzerland, and the Indian National Science Academy, New Delhi, India. Male Swiss albino mice, 10–12 weeks old, 30–36 g, were selected from an inbred colony maintained under controlled conditions of temperature ($23 \pm 2\,^{\circ}C$), humidity ($50 \pm 5\%$) and light (10 and 14 h of light and darkness, respectively). The mice had free access to sterile food and water. Four to six mice were housed in a polypropylene cage containing sterile paddy husk (procured locally) as bedding throughout the experiment. The experiment was approved by the institutional animal ethical committee.

Preparation of the extract

A. conyzoides was identified by Dr G. K. Bhat, Department of Botany, Poorna Prajna College, Udupi, India, and a herbarium specimen is stored in the Department of Pharmacognosy, College of Pharmaceutical Sciences, Manipal, India. The extract of *A. conyzoides* was prepared according to standard protocols. Briefly, the whole plant was shade-dried, powdered and extracted with 95% ethanol using a Soxhlet apparatus. The total ethanolic extract was then concentrated in-vacuo to a syrupy consistency, freeze-dried and stored at $-80\,^{\circ}C$ until further use.

Preparation of the drug solution and mode of administration

The required amount of the 95% ethanolic extract of *A. conyzoides* (ACE) was dissolved in 200 μL of 95% ethanol and suspended in 1% carboxymethylcellulose in physiological saline solution (CMC), before administration. The mice were administered with $0.01\,mL\,g^{-1}$ of CMC (containing an equal amount of ethanol used for ACE preparation) or ACE intraperitoneally.

Determination of acute drug toxicity

The acute toxicity of ACE was determined according to Prieur et al (1973) and Ghosh (1984). Briefly, the mice were fasted by withdrawing food and water for 18 h. They were then divided into several groups of 10 mice each. Each group was injected intraperitoneally with various doses (25, 50, 100, 125, 250, 500, 750, 1000, 2000, 2500 and $3000\,mg\,kg^{-1}$) of the freshly prepared ACE solution. The mice were provided with food and water immediately after the drug administration. Mortality was observed up to 14 days after drug treatment. The treatment of mice with the various doses of ACE did not induce death during the period of the study and hence it was considered safe for administration.

The radioprotective activity of ACE was studied by dividing the mice into two groups as follows: CMC + irradiation: the mice in this group received CMC in saline as described above before irradiation; ACE + irradiation: the mice in this group were injected with ACE before irradiation.

At 30 min after single administration of CMC or ACE, the prostrate and immobilized animals (achieved by inserting cotton plugs in the restrainer) were whole-body exposed to 0, 6, 7, 8, 9, 10 and 11 Gy of ^{60}Co gamma radiation (Theratron, Atomic Energy Agency, Canada) in a specially designed well-ventilated acrylic box. A batch of 10 mice was irradiated each time at a rate of 1.66 Gy min^{-1} at a source to animal distance (surface) of 84.9 cm. The following experiments were then carried out.

Selection of the optimum dose of ACE

To select the optimum dose of ACE for radioprotection, the mice were divided into two groups as described above. The mice in the CMC + irradiation group received CMC, while those in the ACE + irradiation group were administered with 25, 50, 75, 100, 125, 150, 300, 600 and 900 mg kg^{-1} of ACE intraperitoneally, before exposure to 10 Gy of gamma radiation. A dose of 75 mg kg^{-1} ACE was found to be the optimum radioprotective dose and therefore further experiments were carried out using this dose.

Radioprotective effect of ACE

To determine the radioprotective effect of ACE, the mice were divided into two groups as described above. One group of mice received CMC, while the other group was injected with 75 mg kg^{-1} ACE before exposure to 6, 7, 8, 9, 10 and 11 Gy of gamma radiation. The mice in both groups were monitored daily for the development of symptoms of radiation sickness, and mortality for a period of 30 days after irradiation. The death of animals between 3 and 10 days after irradiation was considered to be due to gastrointestinal damage, while death between 11 and 30 days after irradiation was due to damage to haematopoietic organs. Percentage survival was calculated and plotted against the radiation dose. The dose reduction factor was calculated by the method of Miller & Tainter (1944) as follows: dose reduction factor = LD50 of the ACE + irradiation group/LD50 of CMC + irradiation group.

1,1-Diphenyl-2-picryl hydrazyl (DPPH) scavenging activity of ACE in-vitro

The principle of the reduction of DPPH free radical is that the antioxidant reacts with the stable free radical, DPPH, and converts it to 1,1-diphenyl-2-picryl hydrazine. The ability to scavenge the stable free radical DPPH is measured by a decrease in the absorbance at 517 nm (Sreejayan & Rao 1996; Mensor et al 2001).

Aliquots of various concentrations of ACE dissolved in ethanol were mixed with 2 mL of 0.05 mM DPPH solution (in ethanol). Following 20 min of incubation at 37 °C, the absorbance at 517 nm was measured with a UV-vis spectrophotometer (UV-260, Shimadzu Corp, Japan). The experiment was performed in triplicate.

Statistical analysis

The significance of differences between treatments was determined using the *Z*-test for survival studies (Abramowitz & Stegun 1972). The daily survival analysis was carried out using the Kaplan–Meier equation.

Results

The effect of ACE on the survival of mice exposed to different doses of gamma radiation is shown in Table 1 and Figures 1 and 2. The DPPH scavenging activity of ACE is shown in Table 2.

Effect of ACE on acute toxicity

The administration of 250–3000 mg kg^{-1} ACE to mice did not induce death during the observation period, and mice were free from other symptoms of toxicity. It was therefore concluded that ACE did not have any toxic effects up to a dose of 3000 mg kg^{-1}. The testing of higher doses was precluded owing to drug dissolution problems.

Selection of the optimum dose of ACE

To select the optimum dose of ACE for radiation protection, the mice were treated with 0, 25, 50, 75, 100, 125, 150, 300, 600 and 900 mg kg^{-1} ACE before whole-body exposure to 10 Gy of gamma radiation. The mice were monitored daily for 30 days after irradiation for the development of symptoms of radiation sickness and mortality.

The administration of the various doses of ACE did not cause drug-induced mortality. The first mortality in the CMC + irradiation group was observed on Day 5, and all the mice were dead by Day 22 after irradiation (Table 1). Exposure of mice to CMC + irradiation induced symptoms of radiation sickness, including reduction in food and water intake, irritability, watering of eyes, epilation, weight loss, emaciation, lethargy, diarrhoea, and facial oedema. Pretreatment of mice with the various doses of ACE either delayed or reduced the severity of radiation sickness. The onset of radiation-induced mortality was also delayed in the ACE + irradiation group when compared with the CMC + irradiation group. The longest delay was observed for 150 mg kg^{-1} ACE, where the first death was reported on Day 12 after irradiation (Table 1), followed by 300 and 600 mg kg^{-1}, where the first death occurred on Days 11 and 10 after irradiation, respectively. However, the maximum number of mice survived at a dose 75 mg kg^{-1} ($P < 0.0003$) 30 days after irradiation. Further increases in drug dose resulted in a decline in survival, however it was still significantly higher for 100 ($P < 0.0003$), 125 ($P < 0.001$), 150 ($P < 0.01$), 300

Table 1 Effect of various doses of a 95% ethanolic extract of *Ageratum conyzoides* (ACE) on the survival of mice exposed to 10 Gy of gamma radiation.

ACE (mg kg^{-1})	Mortality (days after irradiation)																													Survivors (%)	Total no. of animals used
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30		
0	–	–	–	–	2	–	–	4	2	–	4	6	–	–	–	–	–	–	–	2	–	4	–	–	–	–	–	–	–	–	0 (0)	24
25	–	–	–	2	–	–	–	2	–	–	2	–	–	–	–	–	1	–	–	2	–	–	–	–	–	–	1	–	–	–	2 (16)	12
50	–	–	–	–	–	2	–	1	–	–	–	2	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	4 (33.3)[b]	12
75	–	–	–	–	–	–	1	2	1	1	–	–	–	–	–	–	–	–	–	–	1	–	–	1	–	–	–	–	–	–	17 (70.8)[d]	24
100	–	–	–	–	–	–	1	–	1	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	8 (66.7)[d]	12
125	–	–	–	–	–	–	–	1	–	1	–	–	–	–	–	1	–	–	–	–	–	2	–	–	–	–	–	–	–	–	7 (58.3)[c]	12
150	–	–	–	–	–	–	–	–	–	–	–	1	–	1	–	–	2	–	–	–	1	–	–	–	1	–	–	1	–	–	5 (41.6)[b]	12
300	–	–	–	–	–	–	–	–	–	–	2	1	–	1	–	–	1	–	2	–	–	–	–	–	–	–	–	–	–	–	5 (41.6)[b]	12
600	–	–	–	–	–	–	–	–	–	1	–	–	2	–	1	–	1	1	–	–	1	–	–	–	1	–	–	–	1	–	3 (25)[a]	12
900	–	–	–	–	–	–	–	–	2	–	–	1	2	–	–	–	–	1	2	–	–	1	1	–	–	–	–	–	–	–	2 (16)	12

[a] $P < 0.03$; [b] $P < 0.01$; [c] $P < 0.001$; [d] $P < 0.0003$.

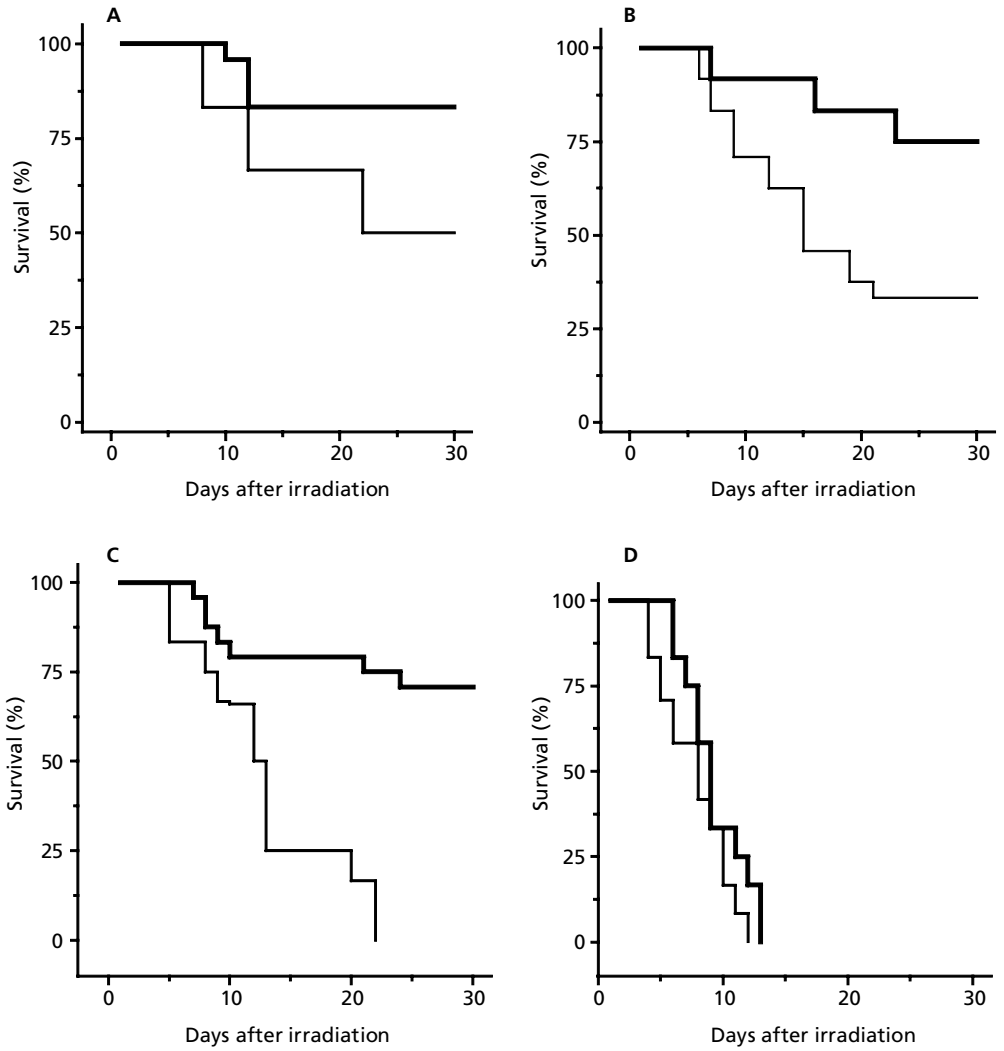

Figure 1 Kaplan–Meier estimate of survival of mice treated with 75 mg kg^{-1} of a 95% ethanolic extract of *Ageratum conyzoides* (ACE) before exposure to various doses of gamma radiation: 8 Gy (A); 9 Gy (B); 10 Gy (C); and 11 GY (D). Thin line, CMC + irradiation; bold line, ACE + irradiation. n = 12 mice per group.

($P < 0.01$) and 600 ($P < 0.03$) mg kg^{-1} ACE when compared with CMC + irradiation (Table 1). A dose of 75 mg kg^{-1} ACE was considered as the optimum radioprotective dose and further studies were carried out using this dose.

Radioprotective effect of ACE

The results are expressed as percentage survival after exposure to various doses of gamma radiation (Figure 1). The radioprotective effect of ACE was studied in mice treated with 75 mg kg^{-1} ACE before exposure to 6, 7, 8, 9, 10 and 11 Gy of gamma radiation. The mice in the CMC + irradiation group exhibited signs of radiation sickness within 2–4 days after exposure 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 symptoms of radiation sickness. The main symptoms included reduction in food and water intake, irritability, epilation, watering of eyes, weight loss, emaciation, lethargy, diarrhoea, and ruffling of hair. Facial oedema was also observed in a few mice between 1 and 2 weeks after exposure to 9, 10 and 11 Gy of gamma radiation. A few mice exhibited paralysis (3%) and difficulty in locomotion during the second week after exposure. The severity of the symptoms increased with an increase in radiation dose.

The whole-body irradiation of mice with up to 7 Gy of gamma radiation did not induce mortality in either group. With increasing doses of radiation, the survival declined in a dose-dependent manner until a nadir in survival was reached at 11 Gy, at which no survivors were reported beyond 22 days after irradiation. With the increase in the exposure dose, the onset of mortality was also advanced.

Pretreatment of mice with 75 mg kg^{-1} ACE delayed or reduced the severity of radiation sickness and

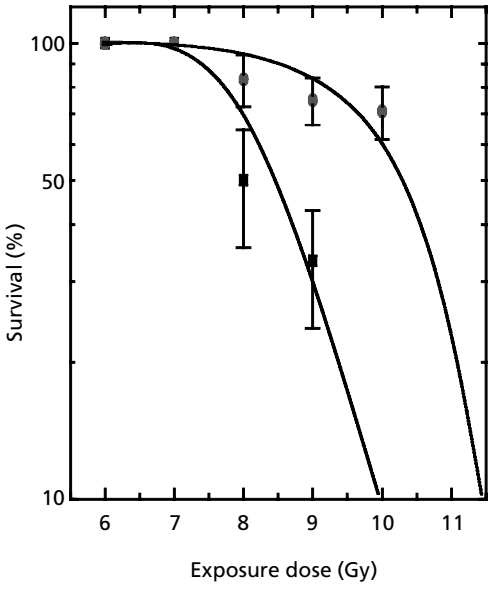

Figure 2 Effect of 75 mg kg^{-1} of a 95% ethanolic extract of *Ageratum conyzoides* (ACE) on the 30-day survival of mice exposed to various doses of gamma radiation. CMC + irradiation (■); ACE + irradiation (●) group. Error bars represent 95% confidence limits.

Table 2 Effect of various doses of a 95% ethanolic extract of *Ageratum conyzoides* (ACE) on 1,1-diphenyl-2-picryl hydrazyl (DPPH) scavenging activity in-vitro.

ACE dose (μg mL^{-1})	Percent DPPH scavenging (mean ± s.e.m.)
7	2.97 ± 0.06
15	5.83 ± 0.03
32.10	8.02 ± 0.03
62.50	12.32 ± 0.13
125	32.70 ± 0.61
250	71.49 ± 0.30
500	80.93 ± 0.35
1000	86.14 ± 0.44

radiation-induced mortality when compared with the CMC + irradiation group (Figure 1). This delay in the onset of mortality was by almost 2–3 days in the ACE + irradiation group when compared with the CMC + irradiation group. The gastrointestinal related deaths were less when compared with the CMC + irradiation group for all exposure doses. ACE pretreatment also reduced the bone marrow related deaths (Figure 1). ACE pretreatment reduced the 30-day mortality by 1.5-, 2.25- and 2.1-fold, for 8 ($P < 0.001$), 9 ($P < 0.0001$) and 10 Gy ($P < 0.001$) of gamma radiation, respectively (Figure 2). The dose reduction factor was 1.3.

DPPH scavenging activity by ACE in-vitro

ACE scavenged DPPH in a concentration-dependent manner in-vitro. The lowest concentration of 7 μg mL^{-1} ACE caused 2.90% scavenging of DPPH. There was a sudden elevation in the DPPH scavenging activity at 250 μg mL^{-1}, where 71.49% DPPH scavenging was observed. Thereafter, this increase was gradual until peak DPPH scavenging (86.85%) was observed at 1000 μg mL^{-1} (Table 2).

Discussion

Since the discovery of the deleterious effects of radiation, efforts have been directed to alleviate radiation-induced damage. Several synthetic compounds have been used as radioprotectors and thiol compounds initially looked very promising. However, their practical applicability remained limited owing to their high toxicity at the optimum protective dose. Plants and natural products may prove useful in this regard; however, information on their radioprotective ability is limited. The present study was undertaken to evaluate the radioprotective effect of ACE in mice whole-body exposed to different doses of gamma radiation.

There is an urgent requirement to screen for new non-toxic radioprotectors to protect the general public from the threat of nuclear terrorism and particularly for cancer patients undergoing radiation treatment. Despite screening of several synthetic compounds for radioprotective activity, no single compound has emerged as a good radioprotector (Sweeny 1979). Plants have formed the basis of several useful drugs for the treatment of various ailments. Therefore, screening of natural products presents a major avenue for the discovery of new radioprotective drugs.

Single whole-body exposure of mice to ionizing radiation results in a complex set of symptoms, the onset, nature and severity of which are a function of both the total radiation dose and radiation quality. At the cellular level, ionizing radiation can induce damage in biologically important macromolecules such as DNA, proteins, lipids and carbohydrates in the various organs. While some damage may be expressed early, other damage may be expressed over a period of time, depending on cell kinetics and the tolerance of the tissues to radiation. 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 a life-supporting function, but the gastrointestinal epithelium and bone marrow progenitor cells are crucial for life and any damage to these cells will drastically impair the normal physiological processes, with adverse effects on survival of the exposed individual. The gastrointestinal epithelium is less sensitive than bone marrow progenitor cells, but as the cell transit time is quick, it is expressed earlier than the haemopoietic syndrome. In mice, death within 10 days after irradiation is due to gastrointestinal damage (Bond et al 1965; Jagetia et al 1993, 2002, 2003; Uma Devi et al 1999; Jagetia & Baliga 2002). The bone marrow stem cells are more sensitive to radiation damage than the intestinal crypt and the haemopoietic syndrome occurs at lower doses and is

manifested as haemopoietic stem cell depletion, followed by the depletion of mature haemopoietic and immune cells. Death between 11 and 30 days after irradiation is due to damage to the haemopoietic system (Bond et al 1965; Jagetia et al 1993, 2002, 2003; Uma Devi et al 1999; Jagetia & Baliga 2002).

Administration of different doses of ACE resulted in a dose-dependent decline in the radiation-induced mortality up to a dose of 75 mg kg^{-1}, where the greatest decline in the radiation-induced mortality was observed. Previous studies on radioprotection have shown that a test agent acts only at a particular dose, above which it may not be protective and may even be toxic (Thomson 1962). Radioprotective substances have been reported to have a cardinal dose, above and below which protection may not be significant (Thomson 1962; Ganasoundari et al 1997b; Jagetia & Baliga 2002; Jagetia et al 2002, 2003). The reason may be that after a particular concentration, a compound, instead of being an antioxidant may act like a pro-oxidant, inducing toxic symptoms and resulting in death.

ACE pretreatment reduced the symptoms of radiation sickness and mortality induced by various doses of radiation. Similarly, other extracts from plants such as *O. sanctum*, *P. ginseng*, *Gingko biloba* and *M. arvensis* have been reported to have radioprotective effects (Jagetia et al 1986; Zhang et al 1987; Hahn et al 1994; Ganasoundari et al 1997a; Uma Devi et al 1998, 1999; Alaoui-Youssefi et al 1999; Jagetia & Baliga 2002).

The pattern of survival in the ACE + irradiation group was similar to that of the CMC + irradiation group, except that mortality was delayed. This clearly indicates the effectiveness of ACE in arresting gastrointestinal related death, where the number of survivors at doses of 75, 100 and 125 mg kg^{-1} ACE was higher than that of the irradiated control. This reduction in gastrointestinal related death may be due to protection of the intestinal epithelium, allowing proper absorption of nutrients. The ethanolic extract of *A. conyzoides* has been shown to protect the gastric mucosa of Wistar rats against ethanol-induced gastric ulceration (data not shown). Pretreatment of mice with ACE significantly reduced the bone marrow related deaths in the ACE + irradiation group, especially at doses of 75, 100 and 125 mg kg^{-1}, where a significant increase in survival was observed. This increase in 30-day survival may be due to the protection afforded by ACE to the stem cell compartment, which continued to supply the requisite number of cells in the survivors. A similar observation has been made for copper glycinate, orientin, vicenin, *Boerhaavia diffusa*, *M. arvensis* and *O. sanctum* (Jagetia et al 1986, 1993; Jagetia & Ganapathi 1990; Ganasoundari et al 1997a; Thali et al 1998; Uma Devi et al 1998, 1999; Jagetia & Baliga 2002).

The exact mechanism of radioprotective activity of ACE is not known. However, it is plausible that scavenging of reactive oxygen species by ACE may have played an important role in providing the protection against radiation-induced mortality. This is supported by the concentration-dependent scavenging of DPPH, a stable free radical, by ACE in the present study. Quercetin, a flavonoid present in the ACE (Gonzalez et al 1991), has been reported to scavenge hydroxyl and superoxide free radicals. Flavonoids such as 5′-methoxynobiletin, linderoflavone B and 5,6,7,3′,4′,5′-hexamethoxyflavone, present in *A. conyzoides*, have been reported to increase antioxidant enzymes in-vivo (Quijano 1980; Vyas & Mulchandani 1988; Aruna & Shivaramakrishnan 1990; Gonzalez et al 1991; Pari et al 1998). Similarly, eugenol, which is also present in ACE, has been reported to be a good antioxidant (Vidya & Devraj 1999). The triterpenes, eugenol and flavonoids, which are present in ACE, are good antioxidants and modulators of xenobiotic enzymes, especially phase II enzymes such as glutathione-*S*-transferase and glutathione (Aruna & Shivaramakrishnan 1990). The depletion of glutathione has been implicated as one of the causes of radiation-induced damage, while increased levels of GSH are responsible for radioprotective action (Revesz 1985). Therefore, the radioprotective activity of ACE may also be owing to increased antioxidant status and stimulation of the immune system.

Conclusion

ACE protected against radiation-induced mortality in mice and the optimum protective dose was 75 mg kg^{-1}, at which a dose reduction factor of 1.3 was obtained. The exact mechanism of radioprotection is not known, however ACE may have protected against radiation-induced damage by scavenging radiation-induced reactive oxygen species, increasing glutathione levels or by immunomodulation. Further studies are underway to study the exact mechanism of action and to isolate the active principles involved.

References

Abramowitz, M., Stegun, I. A. (1972) *Handbook of mathematical functions*. Dover Publications, Inc., New York

Alaoui-Youssefi, A., Lamproglou, I., Drieu, K., Emerit, I. (1999) Anticlastogenic effects of *Ginkgo biloba* extract (EGb 761) and some of its constituents in irradiated rats. *Mutat. Res.* **445**: 99–104

Aruna, K., Shivaramakrishnan, V. M. (1990) Plant products as protective agents against cancer. *Ind. J. Exp. Biol.* **28**: 1008–1011

Bond, V. P., Fliedner, T. M., Archambeau, J. O. (1965) *Mammalian radiation lethality*. Academic Press, New York

Ekundayo, O., Sharma, S. Rao, E. V. (1988) Essential oil of *Ageratum conyzoides*. *Planta Med.* **54**: 55–57

Ganasoundari, A., Uma Devi, P., Rao, M. N. A. (1997a) Protection against radiation induced chromosome damage in mouse bone marrow by *Ocimum sanctum*. *Mutat. Res.* **373**: 271–276

Ganasoundari, A., Zare, S. M., Uma Devi, P. (1997b) Modification of bone marrow radiosensitivity by medicinal plants extracts. *Br. J. Radiol.* **70**: 599–602

Ghosh, M. N. (1984) Toxicity studies. In: Ghosh, M. N. (ed.) *Fundamentals of experimental pharmacology*. Scientific Book Agency, Calcutta, pp. 153–158

Githens, T. S. (1948) *Drug plants of Africa*. African Handbooks Series, Vol. 8. University of Pennsylvania, Philadelphia

Gonzalez, A. G., Aguiar, Z. E., Grillo, T. A., Luis, J. G., Rivera, A. Calle, J. (1991) Methoxyflavones from *Ageratum conyzoides*. *Phytochemistry* **30**: 1269

Hahn, S. M., Krishna, M. C., Samuni, A., Degraff, W., Cuscela, D. O., Johnstone, P., Mitchell, J. B. (1994) Potential use of nitroxides in radiation oncology. *Cancer Res*. **54**: 2006s–2010s

Jagetia, G. C., Baliga, S. M. (2002) Influence of the leaf extract of *Mentha arvensis* Linn. (mint) on survival of mice exposed to different doses of gamma radiation. *Strahlenther. Onkol*. **2**: 91–98

Jagetia, G. C., Ganapathi, N. G. (1989) Inhibition of clastogenic effect of Liv 52 in bone marrow of mice. *Mutat. Res*. **224**: 507–510

Jagetia, G. C., Ganapathi, N. G. (1990) Effect of copper glycinate on the radiation induced micronuclei formation in mice bone marrow. *Radiat. Environ. Biophys*. **29**: 115–118

Jagetia, G. C., Uma Devi, P., Singatgeri, M. K., Singh, N., Kohli, R. (1986) Radiation modifying effect of *Ocimum sanctum* mouse survival studies. Proceedings of the 56th Annual Session of the National Academy of Science India 40

Jagetia, G. C., Ganapathi, N. G., Unnikrishnan, M. K. (1993) Copper glycinate protects mice exposed to various doses of gamma radiation. *Strahlenther. Onkol*. **5**: 323–328

Jagetia, G. C., Baliga, S. M., Malagi, K. J., Sethukumar Kamath, M. (2002) The evaluation of the radioprotective effect of triphala (an Ayurvedic rejuvenating drug) in the mice exposed to γ-radiation. *Phytomedicine* **9**: 99–108

Jagetia, G. C., Baliga, S. M., Aruna, R, Rajanikant, G. K, Jain, V. (2003) Effect of abana (a herbal preparation) on the radiation-induced mortality in mice. *J. Ethnopharmacol*. **86**: 159–165

Katsuri, T. R., Manithomes, T. M. (1967) Essential oil of *Ageratum conyzoides*: isolation and structure of two new constituents. *Tetrahedron Lett*. **27**: 2573

Kissman, G., Groth, D. (1993) *Plantas infestantes e nocivas*. Basf Brasileira, São Paulo

Kong, T., Ah-N, G., Yu, R., Chen, C., Mandlekar, S., Primiano, T. (2000) Signal transduction events elicited by natural products: role of MAPK and caspase pathways in homeostatic response and induction of apoptosis. *Arch Pharm Res*. **23**: 1–16

Mensor, L. L., Menezes, F. S., Leitao, G. G., Reis, A. S., dos Santos, T. C., Coube, C. S., Leitao, S. G. (2001) Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytother. Res*. **15**: 127–130

Miller, L. C., Tainter, M. L. (1944) Estimation of the ED_{50} and its error by means of logarithmic-probit graph paper. *Proc. Soc. Exp. Biol. Med*. **57**: 261

Negrelle, R. R. B., Sbalchiero, D., Cervi, A. C. (1988) *Espécies Vegetais utilizadas na terapêutica popular no município de Curitiba, Paraná, Brasil*. Universudade Federal do Paraná, Curitiba

Oliveira, F., Akisue, M. K., Garcia, L. O. (1993) Caracterização farmacognóstica da droga e do extrato fluído de mentrasto, *Ageratum conyzoides* L. *Lecta* **11**: 63–100

Pari, K., Rao, P. J., Subrahmanyam, B., Rasthogi, I. N., Devakumar, C. (1998) Benzofuran and other constituents of the essential oil of *Ageratum conyzoides*. *Phytochemistry* **49**: 1385

Penna, A. (1921). *Notas sobre plantas brasileiras*. Arajo Penna Filhos, Rio de Janeiro

Prieur, D. J., Young, D. M., Davis, R. D., Cooney, D. A., Homan, E. R., Dixon, R. L., Guarino, A. M. (1973) Procedures for pre-clinical toxicological evaluation of cancer chemotherapeutic agents: protocols of the laboratory toxicology. *Cancer Chemother. Rep*. **4**: 1–28

Quijano, L., Calderson, J. S., Gomez, G. F., Soria, I. E., Rios, T. (1980) Highly oxygenated flavonoids from *Ageratum corymbosum*. *Phytochemistry* **19**: 2439

Revesz, L. (1985) The role of endogenous thiols in intrinsic radioprotection. *Int. J. Radiat. Biol*. **47**: 361–368

Saini, M. R., Kumar, S. Jagetia, G. C., Saini, N. (1984) Effect of Liv 52 against radiation sickness and mortality. *Indian Pract*. **37**: 1133–1138

Saini, M. R., Kumar, S., Uma Devi, P., Saini, N. (1985) Late effects of whole-body irradiation on the peripheral blood of mice and its modification by Liv. 52. *Radiobiol. Radiother*. **26**: 487–493

Sreejayan, N., Rao, M. N. A. (1996) Free radical scavenging activity of curcuminoids. *Arzneimittelforschung* **46**: 169–171

Sweeny, T. R. (1979) *A Survey of compounds from the Antiradiation Drug Development Program of the US Army Medical Research and Development Command*. Government Printing Office, Washington DC

Thali, S., Thatte, U., Dahanukar, S. (1998) The potential of *Boerrhavia diffusa* in radiation induced haemopoietic injury. *Amala Res. Bull*. **18**: 20–22

Thomson, J. F. (1962) *Radiation protection in mammals*. Reinhold Publishing Corporation. New York

Uma Devi, P., Bisht, K. S., Vinita, M. A. (1998) A comparative study of radioprotection by *Ocimum sanctum* flavonoids and synthetic aminothiol protectors in mice. *Br. J. Radiol*. **71**: 782–784

Uma Devi, P., Ganasoundari, A., Rao, B. S. S., Srinivasan, K. K. (1999) In vivo radioprotection by *Ocimum* flavonoids in the survival of mice. *Radiat. Res*. **151**: 74–78

Vidya, N., Devraj, S. N. (1999) Antioxidant effect of eugenol in rat intestine. *Ind. J. Exp. Biol*. **37**: 1192–1195

Vyas, A. V., Mulchandani, N. B. (1988) Polyoxygenated flavone from *Ageratum conyzoides*. *Phytochemistry* **25**: 2625

Yamamoto, L. A., Soldera, J. C., Emim, J. A., Goldinho, R. O., Souccar, C., Lapa, A. J. (1991) Pharmacological screening of *Ageratum conyzoides* L. (Mentrasto). *Mem. Inst. Oswaldo Cruz* **86**: 145–147

Zhang, J., Sigdestad, C. P., Gemmell, M. A., Grdina, D. J. (1987) Modification of radiation response in mice by fractionated extracts of *Panax ginseng*. *Radiat. Res*. **112**: 156–163