Quercetin 3-Rutinoside Facilitates Protection Against Radiation-Induced Genotoxic and Oxidative Damage: A Study in C57bl/6 Mice

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ABSTRACT

Radiation-induced oxidative stress and haematopoietic genomic instability is the major concern during planned or unplanned exposure. Use of the natural phytochemicals is an emerging strategy to prevent from the harmful effects of radiation. In the current investigation, Quercetin 3-Rutinoside (Q-3-R), a polyphenolic bioflavonoid, has been evaluated against gamma radiation (2Gy) induced genotoxic damage and oxidative imbalance in mice. Mice were administered with Q-3-R (10mg/kg body weight) 1hr prior to irradiation and evaluated for its antioxidant potential. Anti-genotoxic potential was assessed in terms of chromosomal aberrations in bone marrow cells. Findings revealed that Q-3-R had very high reducing potential, effectively scavenged 1,1-Diphenyl-2-picryl hydrazyl (DPPH) and hydrogen peroxide radicals, chelated metal ions and inhibited lipid peroxidation in a dose dependant manner. The glutathione (GSH) levels were found elevated ($p < 0.05$), while reduced malondialdehyde (MDA) levels were seen in blood and liver tissues of Q-3-R pretreated mice. Significant ($p < 0.01$) reduction in Reactive Oxygen Species (ROS) levels and radiation induced aberrations (dicentrics, rings, fragments, end to end association, robertsonian translocation) following Q-3-R pretreatment was found in bone marrow cells. The present findings demonstrate that Q-3-R can effectively minimise radiation-induced genotoxic and oxidative damages and can be explored further to be used as a potent radioprotector in humans.

Keywords: Genotoxic; Ionizing radiation; Oxidative imbalance; Radioprotection; Quercetin 3-Rutinoside

NOMENCLATURE

Gy	Gray
hrs	Hours
mg	Milligram
kg	Kilogram
μl	Microliter
ml	Milliliter
M	Molar
^{60}Co	Cobalt 60
min	Minute
BHT	Butylated hydroxy toluene
DMSO	Dimethyl sulfoxide
DTNB	5,5-Dithio-bis-2-nitrobenzoic acid
DPPH	1,1-Diphenyl-2-picryl hydrazyl
DNA	Deoxyribonucleic acid
GSH	Glutathione
MDA	Malondialdehyde
ROS	Reactive Oxygen Species
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid

1. INTRODUCTION

Despite of advancements in medical technologies, use of radiation for treatment of various malignancies during radiotherapy, is associated with toxicities to normal tissues[1]. Synthetic radioprotectors, though having very high efficacy, achieved little success primarily due to associated toxicity. There are few radiation countermeasures like CBLB502, 5-AED, Ex-RAD, HemaMax tocopherol succinate etc., which have reached to advance stages of clinical trials[2]. But till date amifostine (WR-2721), is the only US-FDA approved radioprotective drug, intended to reduce the impact of radiation during radiotherapy in head and neck cancer patients. However, the associated side effects at effective dose, have further limited its use in clinics[3]. The related toxicity of these chemical compounds has directed the researchers for exploring the possibility of developing countermeasures that are safe, effective and non-toxic/less toxic.

It is well known that cellular effect of ionizing radiation largely depends on the generation of reactive oxygen species (ROS)[1]. Scavenging of ROS is the most common mechanism rendered by radioprotective agents to protect normal tissues against radiation injuries[4]. Dietary antioxidants are known to maintain the cellular oxidative homeostasis by scavenging the damaging ROS. Moreover, natural antioxidants such as vitamin A, C, E, melatonin and other herbal products, are considered non-toxic/minimally toxic and widely accepted by humans. These natural antioxidants are capable of acting through multiple pharmacological mechanisms, hence under consideration as a useful alternative radioprotectors/mitigators[4].

Radiation-induced genomic instability to the normal tissues is the most serious concern during radiotherapy for healthy survival of patients[5]. Radiation instigates damage to the genetic material or can induce carcinogenesis or mutation at low doses which may be transmitted to the subsequent generation and results in genetic diseases[5]. DNA is the principle target for biologic effects of radiation. Radiation-induced double strands breaks (DSBs) lead to chromosomal aberrations, which are produced by misrepairing of DNA double strand break and simultaneously affect many genes to cause malfunction and death of cells[6]. Radiation-stimulated genetic damage and oxidative stress can be alleviated by intervention of the radioprotectors, which have the ability to minimise biological effects of radiation including lethality, carcinogenicity and mutagenecity. Quercetin 3-Rutinoside (Q-3-R), commonly known as rutin is well reported to exhibit anti-genotoxic potential in irradiated human lymphocytes[7]. Oral administration of Q-3-R in combination with quercetin has been reported to ameliorate radiation-induced micronuclei formation and DNA damage[8]. Q-3-R has also proved its anti-genotoxic effect against anticancer drug Methotrexate in mice model[9]. Q-3-R, a polyphenolic natural flavonoid, is documented to bear the strong antioxidant and anti-inflammatory potential[10]. It is being used as antimicrobial, antifungal, antimutagenic and antiallergic agent in conventional medicine system. Q-3-R, having abundance of antioxidant properties, possesses different protective effects against neurodegenerative disorders, hepatic dysfunction, cardiovascular diseases, skin cancer and various vascular disorders related to capillary permeability and fragility[11].

We have observed strong radioprotective potential of Q-3-R in combination with podophyllotoxin to provide protection to murine hematopoietic, gastrointestinal and pulmonary systems exposed to lethal dose of gamma radiation[12-14]. The combination has been reported to extend >85 per cent survival in lethally irradiated mice. The key compound podophyllotoxin attenuated DNA damage and activated DNA repair pathway due to transient cell-cycle arrestation at G2/M phase and rutin helped in healthy survival of lethally irradiated mice by scavenging free radicals and inhibiting inflammation[15]. We were further interested to explore the potential of Q-3-R alone to reduce the oxidative imbalance and genotoxic stress induced by gamma radiation at a sublethal moderate gamma radiation dose (2Gy). In the current study, detailed *in vitro* and *in vivo* assays were used to establish the antioxidant potential of Q-3-R followed by anti-genotoxic evaluation in bone marrow cells of C57bl/6 mice.

2. MATERIALS AND METHODS

Q-3-R (CAS Number: 207671-50-9), was obtained from Sigma Aldrich (St. Louis, MO, USA) and assessed for its antioxidant (*in-vitro* and *in-vivo*) and anti-genotoxic potential.

2.1 Evaluation of Antioxidant Potential of Q-3-R (*in vitro* assays)

2.1.1 Total Reducing Power

The reductive potential of Q-3-R was determined by following Oyaizu (1986)[16] method. Briefly, different concentrations of test samples were mixed with 200 μl phosphate buffer (0.2M, pH-6.5) and potassium ferricyanide (0.1 %), incubated at 50 °C for 20 min and mixed with 250 μl TCA (10 %). After centrifugation, the supernatant was mixed with 500 μl distilled water and 100 μl ferric chloride (0.1 %), incubated at 30 °C for 10 min and absorbance was recorded by spectrometer at 700 nm.

2.1.2 Free Radical Scavenging Potential

The free radical scavenging activity of Q-3-R was analysed by Koleva *et al.* (2002)[17] method. Various concentrations of samples were mixed with 1 ml methanolic solution of DPPH (0.1 mM). The samples were shaked properly, incubated in dark for 30 min and measured at 517 nm. The percentage inhibition was calculated by the formula: (A0-A1)/A0]×100, where A0 and A1 indicate the absorbance of control and test samples, respectively.

2.1.3 Hydrogen Peroxide (H_2O_2) Radicals Scavenging Potential

Hydrogen peroxide radicals scavenging property was estimated by Ruch *et al.* (1989)[18] method. Briefly, to various concentrations of test sample/standards (0.5 ml), 1 ml H_2O_2 (40 mM) was added and absorbance was measured at 230 nm against a blank.

2.1.4 Metal Chelation Activity

Benzie and Strain (1996)[19] method was used for estimation of metal chelating activity of Q-3-R. In brief, 1 ml o-phenanthroline (0.05 %) dissolved in methanol and 2 ml ferric chloride (200 μM) was added to different concentrations of test samples. After incubation at room temperature for 10min, absorbance of the samples was measured at 510 nm. Metal chelating activity of Q-3-R was calculated in terms of percent scavenging and compared with quercetin, α-tocopherol and BHT.

2.1.5 Anti Lipid-Peroxidation Activity

Buege and Aust (1978)[20] method was employed for estimating anti-lipid peroxidation property of Q-3-R. Briefly, brain tissues of mice were excised and homogenised (10%w/v) with 0.15M KCl. Brain homogenate (0.5 ml) was added to different concentrations of samples followed by incubation at 37 °C for 30 min. After incubation, the samples were mixed with 2 ml of TCA (15 % w/v)-TBA (0.37 % w/v) solution, boiled, cooled, centrifuged and absorbance of supernatants was recorded at 535 nm to calculate percent inhibition of lipid peroxidation.

2.2 *In Vivo* Studies

2.2.1 Preparation of Q-3-R Formulation

Q-3-R formulation, used in the current study, was prepared by dissolving Q-3-R (10 mg/kg body weight) in DMSO. After complete dissolution of Q-3-R in DMSO, distilled water was added so that the final concentration of DMSO in the formulation is 10 per cent. The formulation was injected (100 μl) in single dose in experimental mice intramuscularly, 1 hr prior to 2Gy total body radiation exposure.

2.2.2 Animals and γ-ray Irradiation

C57bl/6 female mice (8-10 weeks old and 25±3 g body weight), obtained from the institute's animal house, were used for the study. The animals were housed in individual cages and maintained under controlled conditions in animal house. The study strictly adheres to the protocols approved by institutional animal ethics committee (IAEC/16/21). Mice were exposed to a single dose of 2 Gy total body irradiation in ^{60}Co gamma chamber (Cobalt Teletherapy Bhabhatron-II, Mumbai, India), at the dose rate of 0.98 Gy/min. Dose calibration was done by institutional radiation physicists using Fricke's dosimetry method[14].

2.2.3 Experimental Design

Randomly selected mice were divided into four groups:

- Control Group: Mice were injected with 0.9 per cent saline (100 μl) intramuscularly and sham irradiated.
- Q-3-R alone Group: Mice were administered with Q-3-R formulation (100 μl) intramuscularly.
- Radiation (2 Gy) Group: In this group animals were exposed to 2 Gy total body irradiation.
- Q-3-R+2 Gy Group: This group was injected with Q-3-R (10 mg/kg body weight) intramuscularly, 1 hr prior to irradiation.

Body weight, food and water intake of all the experimental animals was recorded daily upto 30 days. The animals were sacrificed at different time intervals depending upon the parameters studied. Experiments were repeated thrice.

2.2.4 Hematological Assessment

Blood cells, particularly white blood cells, are highly radiosensitive. To evaluate whether Q-3-R has the ability to protect the peripheral blood cells, hematological studies were carried out. Blood, drawn from cardiac puncture of mice, was diluted in a ratio of 1:20 with Turk's fluid and white blood cells (WBCs) were counted using Neubeaur's chamber under the microscope (Olympus BX-63). Hemoglobin content in all the collected blood samples was measured by using the conventional method (Sahli's hemoglobinometer).

2.2.5 Measurement of ROS Generation by Flow Cytometry

Radiation induces the production of ROS which causes further damage to the biological system. ROS scavenging potential of Q-3-R in the bone marrow cells was measured at 2hrs post treatment by staining the bone marrow cells with 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA) as described earlier[15]. After washing with PBS, the cells (1x10^6) were incubated with 10 μM DCF-DA for 30min at 37 °C and oxidation of dye was measured by using flow cytometer (FACS CALIBUR 3CB, Becton Dickinson Biosciences, USA).

2.2.6 Preparation of Metaphase Chromosomes

Free radicals induced by radiation are responsible for DNA damage which leads to formation of chromosomal aberrations. Yosida and Amano (1965)[21] method was used for preparing chromosome metaphase plates from bone marrow cells by air dry method. Mice were scarified 2 hrs after intraperitoneal injection of colchicine (5 mg/kg body weight). Bone marrow was aspirated, treated hypotonically (0.075 M KCl), incubated for 30 min at 37 °C and fixed in Carnoy's fixative (3:1; methanol: acetic acid). The slides, stained with 5 per cent Giemsa were observed under 100X of Olympus BX-63 microscope. Different types of aberrations like fragments, rings, dicentrics, end to end association, Robertsonian translocations were scored.

2.2.7 Antioxidant Reduced GSH Determination

During overproduction of ROS after radiation exposure, the cellular antioxidant reduced GSH gets oxidised and stabilises the free radicals. Reduced GSH concentration in blood and liver of experimental mice was estimated by following Beutler (1975)[22] method. Briefly, after precipitation of the proteins by precipitating solution (1.67 % metaphosphoric acid+0.2% EDTA+30% NaCl), 4 ml phosphate solution (0.3 M) and DTNB (40 mg in 1 % 100 ml sodium citrate) was added to the supernatants. The absorbance was measured at 412 nm and glutathione concentration was expressed as μg GSH/mg protein.

2.2.8 Lipid Peroxidation Estimation

Radiation generated free radicals causes peroxidation of the lipid of cell membrane, leading to change in their structure and functionality. Lipid peroxidation in terms of MD) was measured in mice liver according to Buege and Aust (1978)[20] method. Briefly, the reaction mixture, prepared by mixing the tissue homogenates in TCA (15 %) –TBA (0.37 %) solution, was bolied and centrifuged. Absorbance of supernatnats was recorded at 535 nm, MDA concentration was calculated by using an extinction coefficient of 1.56×10^5 M^{-1} Cm^{-1} and expressed as nm MDA/mg protein.

2.3 Statistical Analysis

The results of *in vitro* and *in vivo* assays were analysed by using Student's t-test. Experimental results were presented as mean±SE of three independent experiments. The statistically significance difference among the means of groups was analysed by using analysis of variance (ANOVA). Experiments were performed in triplicates with three mice in each group. A value of $p<0.05$ and $p<0.01$ was considered as statistically significant and highly significant, respectively.

3. RESULTS

3.1 Q-3-R Exhibits Strong Antioxidant Potential (in vitro)

To determine the antioxidant potential of Q-3-R, we initially performed a battery of *in vitro* assays and compared with corresponding standards.

3.1.1 Total Reducing Power

Reducing power of Q-3-R compared with standard BHT at range of concentrations (0.05-2 μg) is illustrated by Fig. 1(A). Both Q-3-R and BHT exhibited concentration dependent reduction of ferric ions. At 1.5 μg/ml concentration, Q-3-R showed about 2 fold higher reductive ability (2.3) in comparison to BHT (1.0) at the same concentration.
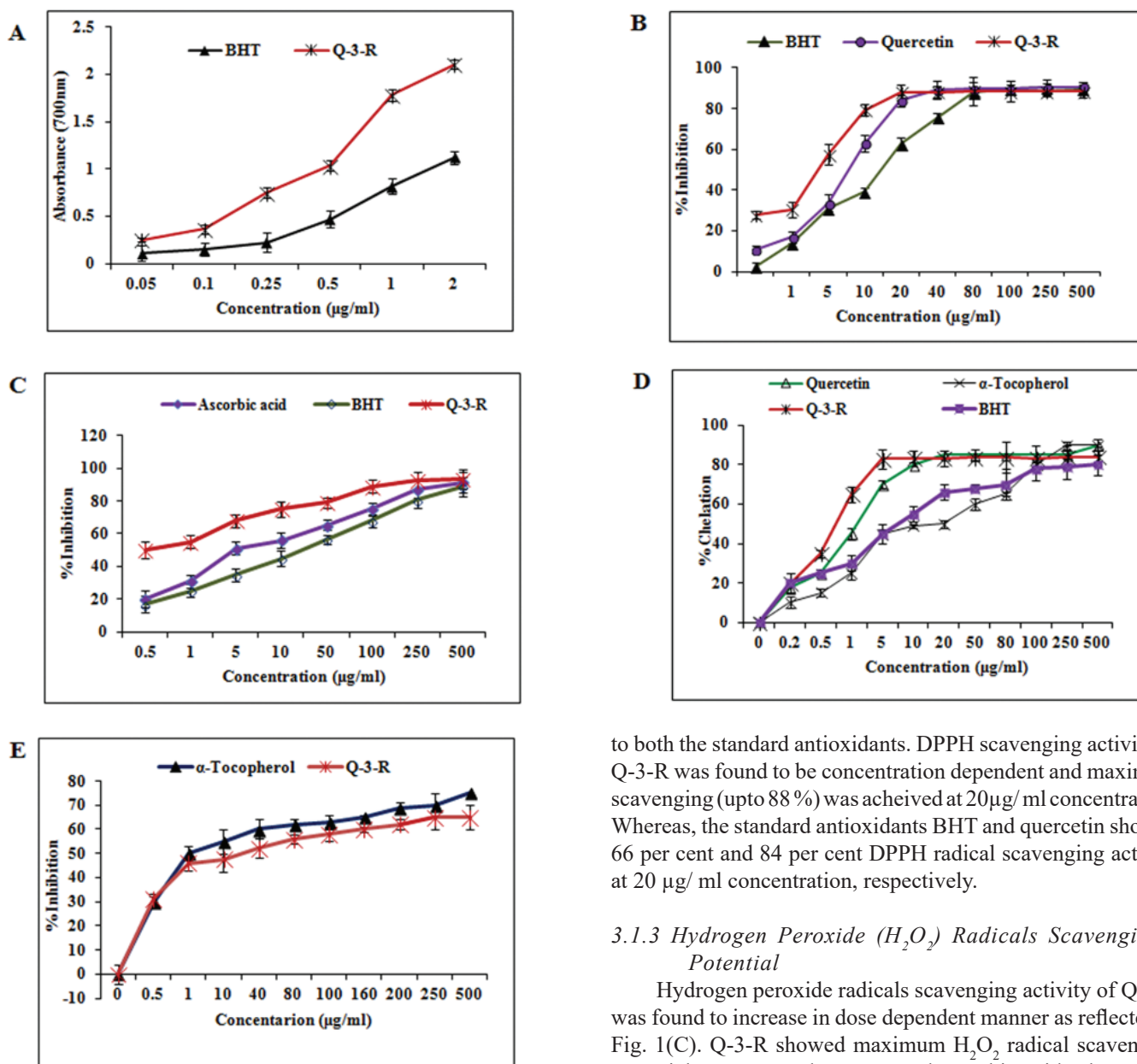
Figure 1. *in vitro* evaluation of antioxidant potential of Q-3-R in comparison to standard antioxidants. (A) Reducing power assay shows higher reductive ability of Q-3-R in comparison to BHT at all the concentrations tested, (B) DPPH radical scavenging activity of Q-3-R compared with standards BHT and quercetin, (C) Hydrogen peroxide radicals scavenging activity of Q-3-R in comparison to ascorbic acid and BHT, (D) Metal chelation ability of Q-3-R at various concentrations compared with BHT, quercetin and α-tocopherol, (E) Anti-lipid peroxidation activity of Q-3-R in comparison to α-tocopherol. Each value represents mean ± SE of three independent experiments.

3.1.2 Free Radical Scavenging Activity

Free radical scavenging activity of Q-3-R was measured by its ability to stabilise DPPH radicals and compared with standards BHT and quercetin. Fig. 1(B) shows that DPPH radical scavenging activity of Q-3-R was high in comparison to both the standard antioxidants. DPPH scavenging activity of Q-3-R was found to be concentration dependent and maximum scavenging (upto 88 %) was acheived at 20µg/ ml concentration. Whereas, the standard antioxidants BHT and quercetin showed 66 per cent and 84 per cent DPPH radical scavenging activity at 20 µg/ ml concentration, respectively.

3.1.3 Hydrogen Peroxide (H_2O_2) Radicals Scavenging Potential

Hydrogen peroxide radicals scavenging activity of Q-3-R was found to increase in dose dependent manner as reflected in Fig. 1(C). Q-3-R showed maximum H_2O_2 radical scavenging potential as compared to BHT and ascorbic acid. The radical scavenging potential of Q-3-R was maximum (89 %) at 100 µg/ ml concentration while ascorbic acid and BHT exhibited 75 per cent and 68 per cent respectively, radicals scavenging at 100 µg/ ml concentration.

3.1.4 Metal Chelation Activity

As depicted in Fig. 1(D), the percent metal chelation activity of Q-3-R was found to be higher in comparison to standard antioxidants BHT, quercetin and α-tocopherol. Q-3-R exhibited its maximum chelation activity (82.9 %) at 5µg/ ml concentration while for quercetin and alpha tocopherol it was 70.0 per cent and 45.0 per cent respectively, at the same concentration (5 µg/ ml).

3.1.5 Anti-Lipid Peroxidation Activity

In the current study, lipid peroxidation was measured in terms of inhibition of thiobarbituric acid reactive species (TBARS). The rate of TBARS inhibition by Q-3-R increased
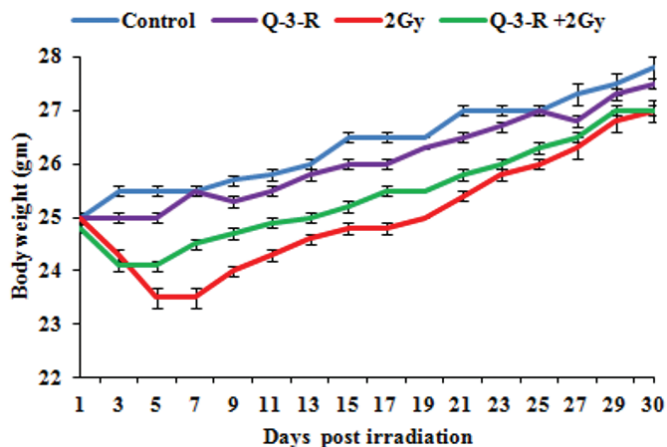
Figure 2. Effect of Q-3-R pretreatment on body weight of 2Gy irradiated mice. Q-3-R was administered 1hr prior to radiation exposure and body weight was monitored daily in differentially treated mice. All experiments were repeated three times. Data are expressed as the mean ± SE, n=3/group.
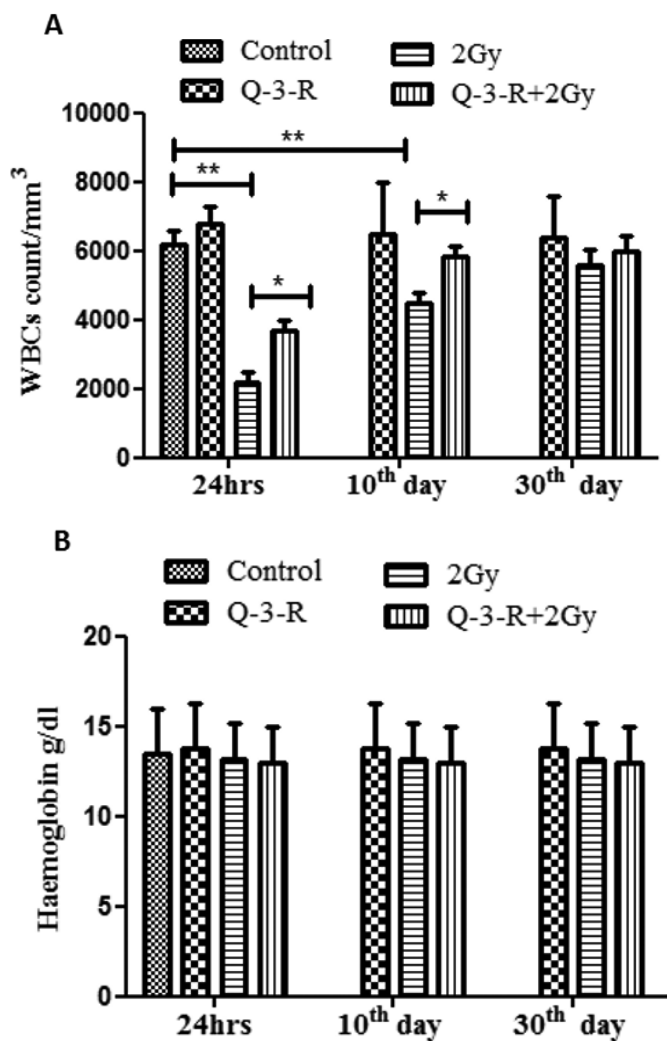

Figure 3. Effect of Q-3-R pre-treatment on (A) WBC and (B) haemoglobin content of 2Gy irradiated mice at different time intervals. Data represented are mean ± SE, n=3/group. The experiments were repeated thrice.

with increasing concentration and was maximum (60 %) at 100 μg/ ml concentration whereas for alpha tocopherol it was 63 per cent at the same concentration. Figure 1(E) shows that TBARS inhibition potential of Q-3-R was comparable to alpha tocopherol.

3.2 *In vivo* Studies

After establishing antioxidant potential of Q-3-R *in vitro*, we further carried out detail *in vivo* studies in C57bl/6 mice to ascertain the role of Q-3-R in minimizing radiation-induced oxidative and genotoxic stress.

3.2.1 Effect on Mice Body Weight

Body weight of mice recorded daily upto 30days, showed nearly 0.5 gm - 1.5 gm fall (2 % - 5 %) till 4-5 days post irradiation, however, the mice started gaining weight and recovered within 7days post exposure (Fig. 2). Mice administered with Q-3-R showed similar trend in body weight, however these mice lost only 0.5 gm - 0.8 gm (2 % - 3 %) body weight post radiation. On day 15, weight of irradiated and Q-3-R pretreated groups was close to controls (Fig. 2). Monitoring of food and water intake in all the treatment group revealed that neither 2Gy irradiation nor Q-3-R treatment had significantly altered the food and water intake of mice (data not shown).

3.2.2 Effect of Q-3-R Pretreatment on Radiation-Induced Haematological Alterations

In all the groups (control, Q-3-R alone, irradiated and Q-3-R pretreated), WBCs circulating in the peripheral blood was estimated at 24 hrs, 10th day and 30th day post treatment and compared with controls. Nearly 3 fold decrease in WBCs count in comparison to controls was observed in irradiated group at 24 hrs post exposure (Fig. 3(A)). Interestingly, mice administered with Q-3-R showed significantly ($p < 0.05$) less decline in WBCs count when compared to irradiated group, indicating protection to blood cells against radiation exposure. On 10th day, in irradiated group WBCs decline was nearly 1.5 fold in comparison to controls. However, Q-3-R pretreated mice showed significantly ($p < 0.05$) improved WBCs count in comparison to irradiated mice at same time interval. WBCs count was nearly comparable to controls in both the groups on 30th day.

Haemoglobin content was also evaluated in all the treatment groups and compared with the controls. However, no significant difference in haemoglobin content was observed in both irradiated and Q-3-R administered groups when compared with controls, at all the studied time intervals thus suggesting that radiation at 2Gy dose has no adverse effect on haemoglobin levels (Fig. 3(B)).

3.2.3 ROS Scavenging Potential of Q-3-R

Figure 4 dipicts nearly 2 fold increase in ROS levels in mice bone marrow cells 2 hrs after exposure to 2 Gy radiation as compared to controls ($p < 0.01$). Administration of Q-3-R before radiation exposure significantly ($p < 0.01$) reduced radiation-induced ROS generation in radiosensitive bone
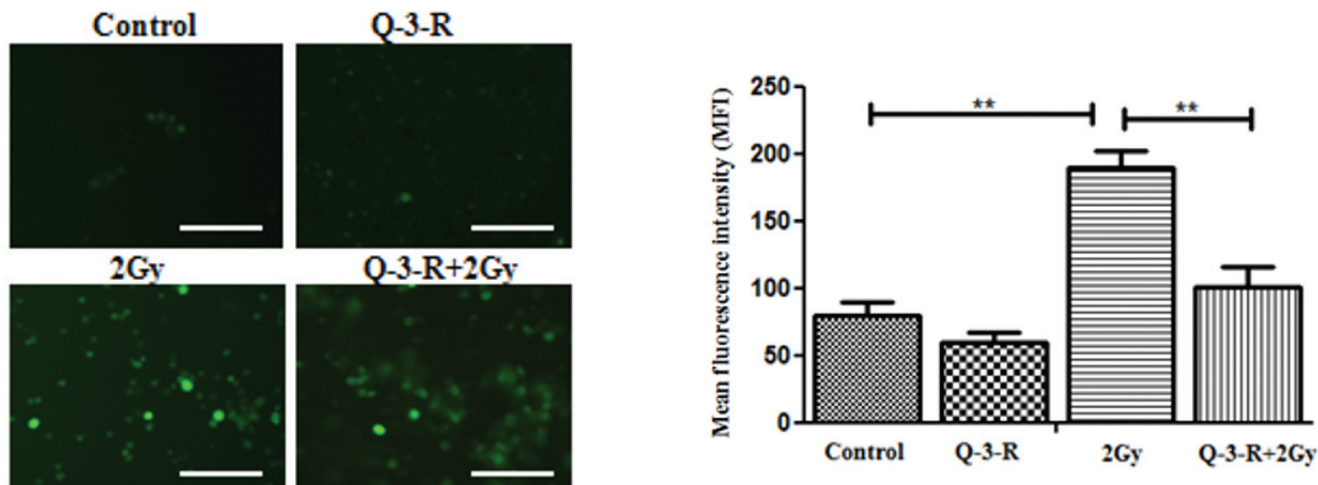
Figure 4. Evaluation of ROS scavenging potential of Q-3-R in mice bone marrow cells. Mice were either irradiated (2 Gy) or administered with Q-3-R 1 hr prior to exposure to 2Gy radiation. ROS generation was measured in bone marrow cells by DCF-DA, 2 hrs after treatment to evaluate the ROS scavenging potential of Q-3-R. Fluorescence microscopic images represent radiation-induced ROS formation in bone marrow cells of differentially treated mice (400X, scale bar 50 µm). Bar graph shows mean flourescent intensity in differentially treated groups. Error bars are SE for n=9. **p<0.01, considered as statistically significant. All the figures are representative of three independent experiments.
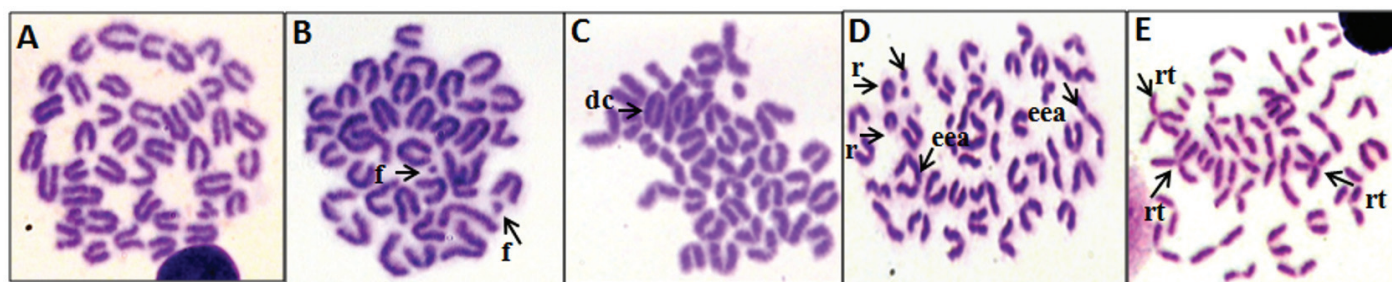

Figure 5. Photomicrograph of bone marrow metaphase plates of mice (100X). Panel A: Control, Panel B-E: Radiation (2Gy) induced different types of aberrations. Arrows indicate fragments (f), rings (r), dicentrics (dc), end to end association (eea), robertsonian translocation (rt).

marrow cells. Imaging of bone marrow cells also showed strong ROS scavenging activity of Q-3-R.

3.2.4 Modulation of Radiation-Induced Genetic Damage by Q-3-R

Total body exposure of 2 Gy gamma radiation in mice led to induction of various kinds of chromosomal aberrations in bone marrow cells of mice at 24 hrs, 10th and 30th day (Fig. 5). The frequency of these aberrations was very high at 24 hrs in irradiated bone marrow cells in comparison to 10th and 30th day in the same group (Table 1). About 58 per cent cells were found to have aberrations after 24 hrs at 2 Gy post exposure in comparison to controls. However, pre-treatment of Q-3-R 1hr prior to irradiation resulted in nearly 50 per cent reduction in number of fragments, dicentrics, end to end association in bone marrow cells at 24 hrs. Robertsonian translocations were 4 times less in these mice in comparison to corresponding irradiated group. On 10th day, in Q-3-R pre-administered mice, fragments and rings were reduced to nearly half as compared to irradiated mice (p<0.01) and no dicentric, robertsonian translocation and end to end associations were observed. Intensity of highly aberrated

plates was also significantly (p<0.01) low in this group on 10th day in Q-3-R pre-treated mice in comparison to corresponding irradiated group, thus indicating that Q-3-R pre-administration can effectively reduce genotoxic effects of radiation. However, on 30th day aberrations were almost comparable to controls in this group (Table 1). Q-3-R alone treatment showed no visible structural change in chromosomes of mice.

3.2.5 Effect of Q-3-R on Reduced GSH Level

Variations in GSH level in blood and liver of experimental mice are depicted in Fig. 6(A) & Fig. 6(B), respectively. Significant (p<0.01) reduction in GSH concentration was noticed in both the organs at 24 hrs in irradiated group. However, on 10th day GSH levels was comparable to controls in this group. Pretreatment of Q-3-R significantly (p<0.05) maintained GSH content in irradiated animals at 24 hrs in both blood and liver. Q-3-R alone did not induce any effect on GSH levels at any time interval.

3.2.6 Effect of Q-3-R on Lipid Peroxidation

Lipid peroxidation (MDA levels) was measured in liver of differentially treated mice by measuring TBARS

Table 1. Effect of Q-3-R pretreatment on bone marrow chromosomes of 2Gy irradiated mice at different time intervals.

Groups	Fragments	Rings	Dicentrics	Robertsonian translocations	End to end associations	Per cent Aberrated plates
Control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Q-3-R	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
2Gy (24hrs)	72.0±8.2 [a]**	16.3±3.6 [a]**	11.2±2.3 [a]**	16.6±1.8 [a]**	19.6±2.5 [a]**	57.8±6.5 [a]**
Q-3-R+2Gy (24hrs)	46.3±3.5 [b]**	12.5±2.0	5.2±1.2 [b]**	4.7±0.5 [b]**	9.7±1.8 [b]**	36.3±3.9 [b]**
2Gy (10th day)	12.5±1.5 [c]**	8.3±2.3 [c]**	1.5±0.2 [c]**	9.4±1.2 [c]*	4.5±0.9 [c]**	34.5±4.1 [c]**
Q-3-R+2Gy (10th day)	5.3±1.1 [d]**	4.3±1.0 [d]**	0.0±0.0 [d]**	0.0±0.0 [d]**	0.0±0.0 [d]**	22.4±2.0 [d]*
2Gy (30th day)	0.0±0.0	0.2±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.10±0.0
Q-3-R+2Gy (30th day)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Values are expressed as mean ± SD of 50 metaphases/ mice from each group of experimental animals. Experiments were performed in triplicates having 3 animals in each group.
[a] 2Gy-24hrs vs. controls, [b]Q-3-R+2Gy-24hrs vs. 2Gy-24hrs, [c]2Gy-10th day vs. controls, [d]Q-3-R+2Gy-10th day vs. 2Gy-10th day. *p<0.05, **p<0.01
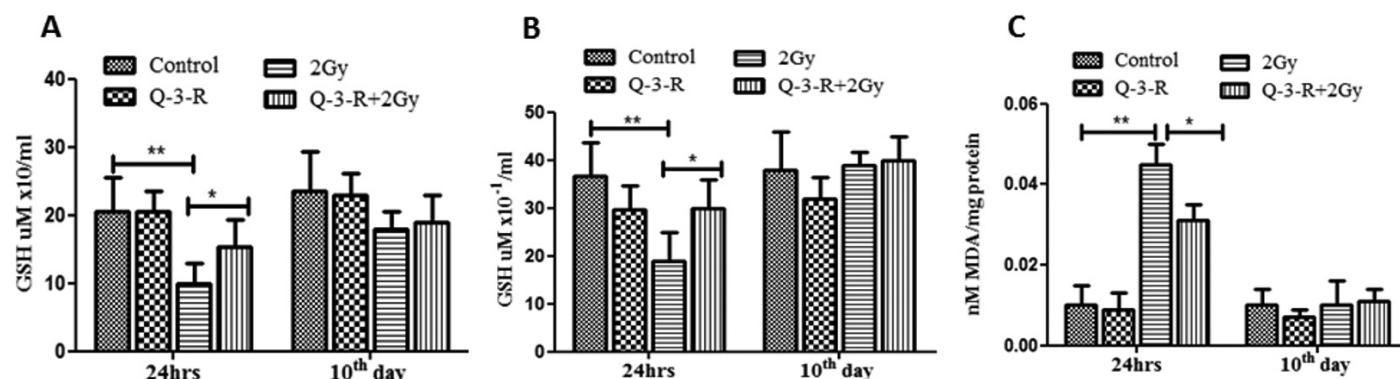

Figure 6. Effect of pretreatment of Q-3-R on GSH and MDA level of 2Gy irradiated mice. Bar graphs (A) and (B) show GSH in blood and liver of differentially treated mice, respectively. GSH present in the sample reacts with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and form yellow coloured product 5-thio-2-nitrobenzoic acid (TNB) which was measured at 412 nm against the reagent blank. Bar graph (C) shows malondialdehyde (MDA) content in liver of differentially treated mice. Lipid peroxidation was evaluated by estimating the stable lipid peroxidation by-product, MDA in tissue homogenates of different treatment groups after 24hrs. The experiment was performed in triplicate with 3 animals in each group. Error bars are SE for n =3. *p< 0.05, **p< 0.01.

concentrations. Total body irradiation of mice to 2Gy radiation dose resulted in nearly 10 fold increase (p<0.01) in MDA level at 24 hrs post exposure (Fig. 6(C). However, administration of mice with Q-3-R prior to 2Gy radiation caused reduction in malondialdehyde level by nearly 2 fold (p<0.05) in liver, when compared to irradiated group (Fig. 6(C). On 10th day MDA level was near to controls in all the treated animals. Administration of Q-3-R alone exhibited no significant alterations in MDA level in liver of treated mice.

4. DISCUSSION

Ionizing radiation, a potent genotoxic agent, inflicts several types of DNA damage in the mammalian cells when the cellular enzymatic and non enzymatic antioxidant systems are inadequate. Radiation-induced DNA double strand breaks (DSB), are highly deleterious and can lead to cell death either by chromosomal reorganisation or direct induction of apoptosis. Alternatively, mis-repaired or unrepaired DSB can lead to mutations or genomic instability in a surviving cell. Hence, development of a safe and effective radioprotector is crucially needed to counter the damaging effect of radiation[23]. Most of the synthetic radio-modifiers developed till date can act by modifying the extent of initial radiation damage to DNA primarily by scavenging the highly reactive oxygen species[2]. However, due to undesired toxicity of these synthetic radioprotectors, research has been directed to explore the natural products/antioxidants/herbal extracts for their protective potential against radiation damage[23].

Flavonoids possess wide range of antioxidant properties due to their associated hydroxyl group, they act as free radicals scavenger, get oxidised and results in formation of more stable and less reactive radicals[24]. Quercetin 3-rutinoside (Q-3-R), a bioflavonoid with strong antioxidant and anti-inflammatory potential, has also been explored for its radioprotective potential

in various *in vitro* and *in vivo* model systems[7, 25-26]. In the current study, we have demonstrated anti-genotoxic effect of Q-3-R in 2 Gy gamma irradiated mice. Administration of Q-3-R, 1 hr prior to radiation, has been found effective in protecting mouse bone marrow cells against radiation-induced chromosome damage as evident by reduction in fragments (f), dicentrics (dc), rings (r), end to end associations (eea), robertsonian translocation (rt) and total aberrated plates (Fig. 5, Table 1). The appearance of significantly less aberrated cells in irradiated bone marrow as observed on 10th day in the current study is possibly due to the fact that chromosome aberrations are lost by nearly 50 per cent with each subsequent cell division[27]. Interestingly, at this time point also, these aberrations were found markedly declined in Q-3-R pretreatment group as compared to irradiated, asserting anti-genotoxic potential of Q-3-R against radiation. Our findings are in line with previous reports, wherein anti-genotoxic effect of rutin in 2 Gy irradiated human lymphocytes was also clearly demonstrated either singly or in combination with quercetin following oral administration[25]. Various *in vitro, ex-vivo* and *in vivo* studies carried out in our group have extensively demonstrated the DNA protecting and anti-genotoxic potential of rutin in combination with lignans[12,28]. DNA protecting potential of Q-3-R may be attributed to its strong free radicals scavenging properties. In the current study also, Q-3-R exhibited strong radicals scavenging potential both in *in-vitro* model systems as well as in radiosensitive murine bone marrow cells. Q-3-R has 15 carbon skeleton consisting of 2 benzene rings (A and B) and oxidation of B ring by free radicals leads to formation of a stable radicals[10]. As shown in the current study and found in the earlier reports[26] also, Q-3-R demonstrates strong radicals scavenging potential in *in vitro* model systems (Fig. 1). Functional hydroxyl groups present in Q-3-R facilitates donation of hydrogen atoms and stabilisation of radiation-induced free radicals species. Irradiation increases the level of redox reactive iron (Fe^{3+}) which participates in Fenton reaction, triggers generation of hydroxyl radicals and further amplifies tissue damage[29]. Radiation-induced hemolysis increases the iron content in the cellular milieu causing amplification of damaging effect[30]. In the current investigation Q-3-R was found to chelate iron more effectively than standard antioxidants (Fig. 1D). The plausible mode of action of herbal radioprotectors is either by removal of free iron from the cellular milieu or by conversion to less toxic ferric state.

In addition, Q-3-R also played a significant role in protecting the cellular antioxidant system, which was otherwise disrupted by radiation exposure. Glutathione maintains cellular redox status as the thiol group present in cysteine moiety of GSH reacts with free radicals and protect cells from oxidative damage[31]. In the present study, GSH level was found declined in blood and liver of irradiated mice due to enhanced utilisation of this cellular antioxidant in attempt to detoxify radiation-generated free radicals. Pre-irradiation administration of Q-3-R could successfully up-regulate/maintain the release of key antioxidants which resulted into minimal damage to these tissues. Radiation-induced ROS also target the lipids of cell membranes and propagates lipid peroxidation chain reactions subsequently leading to cell death. The products of lipid-peroxidation such as malondialdehyde and 4-hydroxynonenal display high biological activities and can destroy DNA, proteins subsequently activate cell death signaling pathways[32]. Interaction of Q-3-R with the polar heads of lipid by formation of hydrogen bond, enhances cell membrane rigidity and protects it from radiation-induced from oxidative damage[33]. Presence of Q-3-R in treated animals had certainly lowered ROS production, led to minimise lipid peroxidation and maintained the balance of antioxidant enzymes. In an earlier report by our group, rutin in combination with lignans had been reported to modulate endogenous antioxidant enzymes in vital organs of lethally irradiated mice[34]. Overall finding from the present study it can be concluded that Q-3-R either by directly scavenging free radicals or by limiting intracellular ROS production by inhibition of enzymes required for ROS generation such as cyclo-oxygenase (COX), xanthine oxidase, mitochondrial succinoxidase, NAD(P)H oxidase etc.[35], successfully maintained the cellular redox balance and minimised the ill effects of radiation.

Nausea, vomiting, dyspepsia are the common symptoms reported in the patients following radiotherapy. Further weight loss during treatment is correlated with compromised immunity and may serve as an important indicator of malnutrition[36]. In the current study we observed that pretreatment of Q-3-R prior to radiation had significantly improved the weight of irradiated mice, indicating that Q-3-R administration has improved nutritional status, thus could prevent weight loss in comparison to irradiated group (Fig. 2).

WBCs are the most important indicator of the hematopoietic damage as the lymphocytes are extremely radiosensitive and serve as a biological dosimeter[20]. Fall in WBCs count following radiation exposure leads to compromised immunity and life-threatening infections. In this study, we observed that Q-3-R pretreatment significantly prevented fall in WBCs count in irradiated mice (Fig. 3), indicating that it could protect radiation-induced hematopoietic damage and can facilitates in
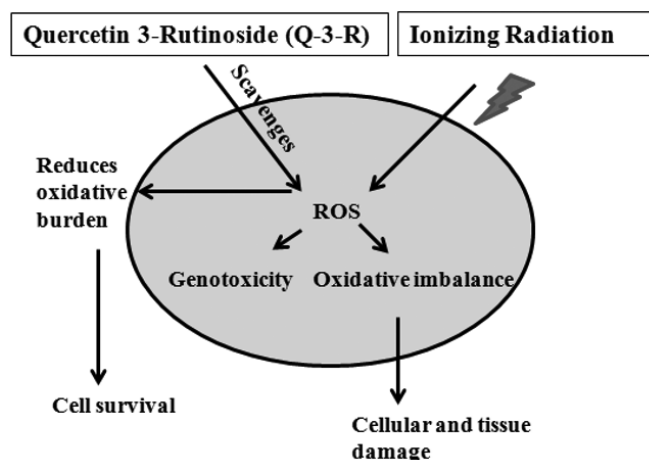

Figure 7. Hypothetical model depicts the role of Q-3-R in minimizing radiation-induced genotoxicity and oxidative stress. Upon exposure to ionizing radiation intracellular ROS generation causes oxidative damage to cells and DNA leading to cell damage/death. Administration of Q-3-R alleviates radiation-induced oxidative stress and genotoxicity by scavenging ROS.

minimizing radiation injuries thus resulting in fast recovery and healthy survival of irradiated mice (2 Gy).

Depletion of free radicals, reduced lipid peroxidation, upregulation/protection of antioxidant defense, protection to peripheral leucocytes by Q-3-R, in this study, have collectively contributed for providing radioprotection (Fig. 7). However, the precise mechanism underlying the protective effect of Q-3-R against radiation-inflicted genotoxic and oxidative damage needs further investigation.

5. CONCLUSION

The present study demonstrates that Q-3-R plays pivotal role in prevention of radiation-induced genotoxic damage. Strong antioxidant effect due to hydrogen or electron-donating potential and ability to up-regulate cellular antioxidants, is responsible for reducing radiation- induced oxidative stress. Q-3-R, being safe as dietary supplement, can be explored as a radioprotective agent for countering radiation-induced tissue injuries.

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