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Quercetin-3-Rutinoside Alleviates Chromosomes Instability Induced by Lethal Dose of Gamma Radiation

Savita Verma

DRDO - Institute of Nuclear Medicine and Allied Sciences (INMAS), Delhi-110 054, India
E- mail: vermasavi@rediffmail.com

ABSTRACT

Radiation-induced genomic instability always remains a major concern for the medical society, following radiation exposure. Hence, development of a safe and effective radioprotector is of considerable interest in case of radiotherapy and radiation emergencies. Quercetin-3-Rutinoside (Q-3-R), a naturally occurring bioflavonoid, has shown promising potential against radiation injuries. The present study reports anti-genotoxic potential of Q-3-R against lethal (9Gy) dose of ionizing radiation. The mice were injected intramuscularly with Q-3-R (10mg/kg b.wt.), 1hr prior to lethal dose of radiation and dissected at 24hrs post treatment. Different kinds of chromosomal aberrations, micronuclei as well as pulverized metaphase and polyploidy were scored in bone marrow cells. Irradiated mice showed significantly ($p<0.001$) increased number of double minutes, fragments, dicentrics, rings, gaps and end to end association. Pulverized metaphases and polyploidy were also found to be elevated in irradiated group. Pretreatment of Q-3-R significantly countered these radiation-induced genetic variations in lethally exposed mice. Data from the present study support the anti-genotoxic potential of Q-3-R even at lethal dose of radiation. Q-3-R, possessing various pharmacological activities and being a safe supplement for treatment of other ailments, can be further explored for its radioprotective potential.

Keywords: Ionizing radiation; Chromosomal aberrations; Pulverized metaphase; Polyploidy; Quercetin 3-Rutinoside

NOMENCLATURE

b.wt.	Body weight
^{60}Co	Cobalt 60
DNA	Deoxyribonucleic acid
DSB	Double strand break
Gy	Gray
hrs	Hours
kg	Kilogram
µl	Microliter
mg	Milligram
min	Minute
ml	Millilitre
Q-3-R	Quercetin-3-Rutinoside
SSB	Single strand break

1. INTRODUCTION

Intentional or unintentional exposure to ionizing radiation during radiotherapy or nuclear accidents poses serious concern for medical society. Radiation-induced genetic risks are one of major challenges faced by radiation biologists and clinicians.[1] Radiation-induced damage to DNA of the cells is manifested in the form of chromosomal aberrations.[2] Radiation can damage DNA either by direct interaction or by production of free radicals in aqueous medium of cells.[3] Damage to DNA by radiation can be induced either by modification of the bases or formation of Single Strand Breaks (SSB) or Double Strand Breaks (DSB).[2] Most of the SSBs induced by ionizing radiation can be repaired via DNA ligation[4]. If both the strands of DNA are broken and well separated, repair again occurs readily as the two breaks are handled separately. If the breaks occur between two opposite strands and are separated by a few base pairs, Double Strands Breaks (DSB) are produced which are the most deleterious lesion induced by ionizing radiation[2] . The interaction between the two DSB may results in cell killing, carcinogenesis or mutation. Unrepaired and misrepaired DSBs are serious threats to the genomic integrity.[5] 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 exerts its deleterious effect mainly by the production of free radicals which are implicated in the process of DNA damage, apoptosis, mutagenesis and carcinogenesis.[7] Hence, it is assumed that the agents capable of scavenging the free radicals can contribute to mitigate the damaging cascade induced by radiation. Radioprotection by the compounds having

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antioxidant potential has been very well documented[7].

Amifostine is the only FDA approved radioprotective drug, though used for only limited application. The drug is being used in clinics for the treatment of xerostomia in head and neck cancer patients undergoing radiotherapy[8]. The underlying radioprotective mechanism of amifostine involves scavenging of free radicals, hydrogen donation, DNA repair[9]. However the associated toxicity allows its application only for limited clinical indications but not for nonclinical uses[10]. Due to inherent toxicity of the synthetic compounds, researchers have directed their interest to the naturally occurring agents having wide spectrum of pharmacological actions. Quercetin-3-Rutinoside (Q-3-R) commonly known as rutin is a bioflavonoid, found in many citrus plants. It has numerous pharmacological potential such as antioxidant, anti-inflammatory, anti-carcinogenic, cardioprotective etc.[11]. The role of Q-3-R against radiation-induced damage in in-vitro and in-vivo model has been well demonstrated[12-13]. Lethal dose of ionizing radiation causes hematopoietic damage which is characterised by loss of circulating cells, anemia and bone marrow suppression[14] . High dose of radiation also damage the gastrointestinal system of the mice leading to loss of electrolytes, dehydration and bacteremia ultimately leading to death of the animals.

In our earlier published reports we demonstrated protection to radiosensitive organs of mice against lethal dose of radiation by administration of Q-3-R, though it was administered in combination with podophyllotoxin[14-15]. In our recently published report, Q-3-R alone has been demonstrated to provide radioprotection to hematopoietic system of mice exposed to low-lethal (7.5Gy) dose of radiation. The present study was undertaken to find the anti-genotoxic potential of Q-3-R against lethal dose of radiation by evaluation of chromosomal aberrations in mice bone marrow cells. Though earlier we have demonstrated the anti-genotoxic potential of Q-3-R against low dose (2Gy) and low-lethal (7.5Gy) dose of radiation, in the present study we were interested to find whether Q-3-R could minimize radiation-induced genetic damage against lethal (9Gy) dose of radiation in mouse model.

2. METHODOLOGY

2.1 Animals and Gamma Irradiation

Healthy male C57BL/6 mice (24±2gm) aged 6-8 weeks were selected for the experiments. The animals were taken from inbred colony INMAS, Delhi, India, where they were maintained at standardised conditions with controlled light (12:12-h light:dark cycle), temperature (22 ± 1 °C) and humidity (50% ± 5%). The animals were kept in polypropylene shoe box type cages and fed with standard food pellet (Amrut Laboratory Animal Feed, Maharashtra, India) and water *ad libitum*. The experimental protocols were approved by Institutional Animal Ethics Committee (INM/IAEC/2016/21; dated: 23-02-2017). Healthy animals were randomly selected and divided into 3 groups (n=6 in each group):

(1) Control: mice were sham irradiated.

(2) Radiation (9Gy): mice were irradiated at 9Gy with total body irradiation.

(3) Q-3-R+9Gy: mice were injected with Q-3-R (10mg/kg b.wt.), intramuscularly, 1hr prior to radiation (9Gy) exposure.

The animals were exposed to radiation in ^{60}Co gamma irradiator (Cobalt Teletherapy, Bhabhatron II, Panacea Medical Technologies Pvt. Ltd., India) at the dose rate of 0.79 Gy/min. Dosimetry was carried out by institutional radiation safety officers.

2.2 Preparation of Q-3-R and Injection

Q-3-R (CAS Number: 207671-50-9, 94% purity) obtained from Sigma Aldrich (St. Louis, MO, USA), was dissolved in 10 per cent DMSO at a concentration of 10 mg/kg b.wt. The dose 10 mg/kg b.wt. was selected based on the available literature[12,24,31]. The mice were injected (100 µl) intraperitoneally with a single dose of Q-3-R, 1hr before irradiation (9Gy).

2.3 Preparation of Metaphase Plates and Slide Scoring

Chromosome aberration study was carried out at 24hrs post irradiation treatment. Metaphase plates from bone marrow cells of mice were prepared by following Yosida and Amano (1965)[16] method. The animals were injected with colchicine (5mg/kg.b.wt.) to arrest metaphases. Two hours after injecting colchicine, the animals were sacrificed and bone marrow was aspirated in PBS. The marrow cells were treated with hypotonic (0.075 M KCl) and fixed in Carnoy's fixative. The slides prepared by air dry method were stained with 5 per cent Giemsa and observed under light microscope (Olympus BX-63 microscope). Slides were coded and scored blind for different types of chromosomal aberrations such as rings, dicentrics, double minutes, fragments, gaps, end to end association, robertsonian translocation as well as cells with pulverized metaphase and polyploidy in 100 metaphases per groups.

2.4 Micronuclei Assay

Micronucleus assay was performed in bone marrow cells of mice according to method described earlier[17]. Briefly, the femur bone of each experimental mouse was aspirated in PBS and centrifuged. The pellet obtained was mixed with few drops of fetal bovine serum and smeared on clean glass slide. The smear was fixed in methanol, stained with May Grunwald-Giemsa and observed under the microscope. A total of 1000 cells were scored and percentage of micronucleated cells was calculated in each experimental group.

2.5 Statistical Evaluation

The results of the experiments were analysed by using student's t-test. One way analysis of variance (ANOVA) with Bonferroni test was performed to determine the significant difference between the test groups. A value of $p<0.01$ was considered statistically significant.

3. RESULTS

3.1 Q-3-R minimizes Radiation-induced Chromosomal Aberrations in Bone Marrow

The metaphases from the control mice did not exhibit any structural or numerical aberration (Fig. 1A). However, the number of all kinds of aberrations (Fig. 1B/D) was raised significantly ($p<0.001$) in 9Gy irradiated mice at 24hrs post irradiation when compared with the control group. Pretreatment of Q-3-R markedly reduced the appearance of these aberrations in bone marrow cells of irradiated mice (Fig. 1E, Table I). A significant decrease was observed in the frequency of fragments, rings, dicentrics, gaps, Robertsonian translocation and end to end association with Q-3-R pretreatment (Fig. 1E). However the appearance of double minutes remained unchanged with Q-3-R pretreatment when compared with irradiated group. The number of cells beyond 24hrs was too less in irradiated bone marrow due to lethal dose exposure, hence aberrations could not be scored at further time points.

3.2 Q-3-R Inhibits Radiation-induced Pulverization and Polyploidy in Bone Marrow

It is well known fact that radiation induces polyploidy and chromosome pulverization.[18] As depicted in Fig. 2A, the number of pulverized metaphase increased significantly ($p<0.001$) in comparison to controls in bone marrow cells of mice exposed to 9Gy lethal radiation. Polyploidy was also found to be enhanced significantly ($p<0.001$) in irradiated group (Fig. 2B). In contrast, pretreatment of Q-3-R markedly ($p<0.001$) reduced pulverisation of chromosomes and polyploidy in lethally exposed mice (Fig. 2C). The number of pulverized metaphase and polyploidy reduced to about 1-1.5 fold in bone marrow of Q-3-R pretreated irradiated mice.

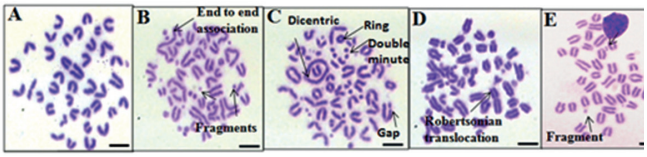

Figure 1. Effect of lethal dose (9Gy) of gamma radiation on cytogenetic damage in mice bone marrow cells. (A) Representative photomicrograph of metaphase plate of control mice, (B) Metaphase from irradiated group shows fragments and end to end association, (C) Metaphase from irradiated group shows presence of ring, dicentrics, gaps and double minutes, (D) Metaphase from irradiated mice shows Robertsonian translocation, and (E) Metaphase from Q-3-R pretreated group shows very few aberrations (magnification 1000X, scale bar 20µm).

3.3 Q-3-R Reduces the Frequency of Micronucleated Cells in Irradiated Bone Marrow

Changes in micronuclei frequency corresponded to that of chromosomal aberration data (Fig. 3A, B). Sham irradiated control group showed 0.15±0.08 micronucleated cells per 100 cells. Whereas, a significant ($p<0.001$) increase was observed in the number of micronucleated polychromatic erythrocytes in bone marrow cells of irradiated mice as compared to control (Fig. 3B). The number of micronucleated cells in irradiated groups was scored 20.3±2.0 per 100 cells. In contrast, administration of Q-3-R, 1hr prior to 9Gy lethal dose could able to reduce ($p<0.01$) the frequency of micronucleated cells (14.0±1.5) in irradiated bone marrow, suggesting its potential to minimize radiation-induced genetic damage.

4. DISCUSSION

The health effects of radiation disaster are potentially horrific and can be carried forward in the form of genetic damage even to the next generation.[19] In Chernobyl radiation accident occurred in 1986, approximately 600 power plant workers received varied doses of radiation. The workers who received very high doses died immediately and other developed cancer at later stages and survived with compromised health.[20] It is well established that ionizing radiation when interacts with the living matter causes the production of free radicals by radiolysis of water.[3] Reactive oxygen species such as hydroxyl radical (•OH), superoxide (O2•−), hydrogen peroxide (H_2O_2) etc. generated after radiation exposure reacts with the cellular macromolecules (lipids, proteins, DNA) and further perpetuate damage.[3] Radiation-induced damage to the genetic material i.e DNA is mainly responsible for the induction of chromosomal aberrations.[2] DNA double-

Table 1. Modifications of radiation-induced chromosomes aberrations in bone marrow cells with Q-3-R pretreatment

Groups	Double Minutes	Fragments	Rings	Dicentrics	Gaps	Robertsonian translocation	End to end association
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	0.0 ±0.0
9Gy	114.5±37.2	1042.0±90.4 [a]**	232.2±20.7 [a]**	100.5±10.6 [a]**	20.0±5.3 [a]**	17.6±0.6 [a]**	116.5±14.5 [a]**
Q-3-R+9Gy	118.6±35.8	659.2±50.9 [b]**	113.6±23.8 [b]**	90.5±6.5 [b]*	10.5±1.2 [b]**	11.8±1.7 [b]*	48.8±3.7 [b]**

Chromosomal aberrations were scored at 24hrs post treatment. Values are expressed as mean ± SD of 100 metaphases mice from each group of experimental animals. [a] 9Gy vs. Control, [b]Q-3-R+9Gy vs. 9Gy, *p<0.01, **p<0.001

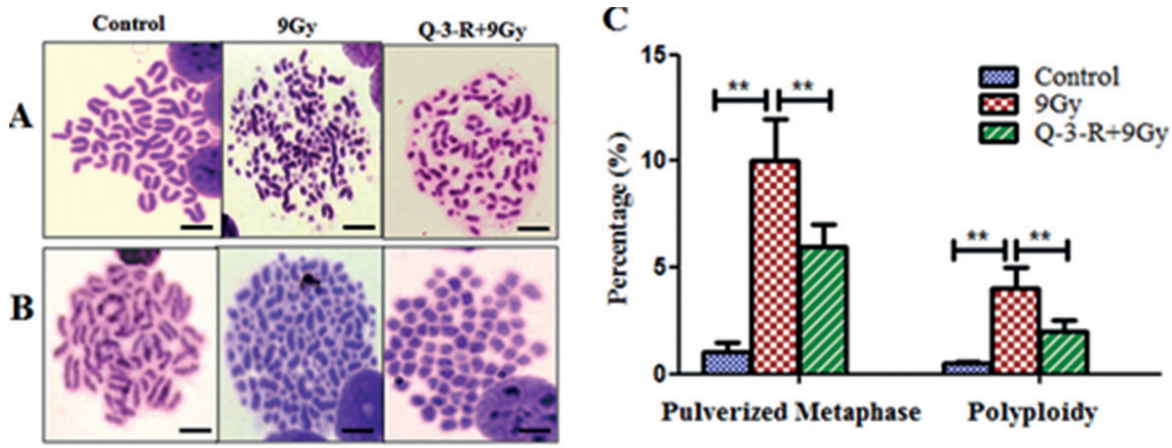

Figure 2. Effect of Q-3-R on pulverized metaphase and polyploidy induced by lethal dose (9Gy) of gamma radiation. (A) Photomicrographs of pulverized metaphase in 9Gy irradiated and Q-3-R pretreated irradiated groups. (B) Photomicrographs of polyploidy in 9Gy irradiated and Q-3-R pretreated irradiated groups (magnification 1000X, scale bar 20µm). (C) Bar graph shows percentage of pulverized metaphase and polyploidy in differentially treated groups. Error bars are SEM for n =6. **p< 0.001.

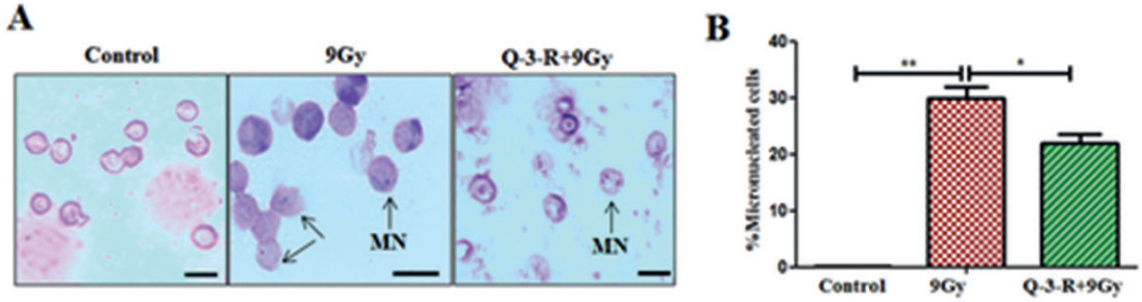

Figure 3. Effect of Q-3-R on micronuclei induced by lethal dose of gamma radiation. (A) Photomicrographs show micronucleated cell (MN) in 9Gy irradiated and Q-3-R pretreated groups (magnification 1000X, scale bar 20µm). (B) Bar graph shows percentage of micronucleated cells in differentially treated groups. Error bars are SEM for n=6. **p< 0.001, p<0.01.

strand breaks created by radiation-generated ROS often result into altered rejoining during the repair process[21]. Misrepaired/unrepaired DNA leads to various types of chromosomal aberrations. Radiation-induced genetic damage may result in mutations, carcinogenesis and progenic transfer of diseases[5]. Therefore there is a need to develop a countermeasure with high efficacy and low toxicity which can minimise the deleterious effects of radiation. Radiation countermeasures can be classified as radioprotectors, radiomitigators and therapeutics. Radioprotectors are administered before the radiation exposure to prevent damage. Radiomitigators are given shortly after the radiation exposure before the symptoms manifest. Whereas, therapeutics are given for recovery when the symptoms appear following radiation exposure. In the nuclear accident scenarios, radioprotectors are useful for the military personnel and the first responders undergoing for rescue or clean-up operation in the fallout fields[22].

Since, ionizing radiation-induced damage is primarily attributed to free radicals generation, antioxidant compounds having free radicals scavenging potential can be promising radioprotectors. The current study reports anti-genotoxic potential of Q-3-R against lethal dose of gamma radiation. Since free radicals are produced within nanoseconds following radiation exposure, therefore to provide the proper radioprotection, the antioxidant compound should be present in the cellular milieu before radiation treatment. In the present study, Q-3-R was administered 1hr prior to radiation exposure for providing efficacious radioprotection. To the best of our knowledge no study has been conducted earlier to determine the anti-genotoxic potential of Q-3-R against lethal dose (9Gy) of gamma radiation. Besides the chromosomal aberrations, radiation exposure leads to error in chromosomal segregation and subsequently leads to formation of micronuclei, chromosome pulverization and polyploidy[18]. In the present investigation radiation-induced genomic modifications were significantly ameliorated by Q-3-R pretreatment. Chromosomal aberrations and other genomic alterations are mediated by radiation-induced generation of ROS/RNS which can cause DNA single-strand or double-strand breaks[23]. Scavenging of these reactive species by Q-3-R effectively reduced DNA damage and subsequently minimised appearance of radiation-induced chromosomal aberrations in bone marrow cells of mice.

Our previous study demonstrated strong anti-oxidant potential of Q-3-R in both in-vivo and in-vitro model

system.[24] Q-3-R being a polyphenolic compound has strong redox potential and plays very pivotal role in stabilizing the radiation-induced free radicals.[25] The antioxidant potential of Q-3-R is mainly attributed to its structural configuration having 10 hydroxyl groups attached to the benzene rings. Q-3-R has 15 carbon skeleton consisting of 2 benzene rings (A and B) and B ring hydroxyl configuration is responsible for donation of hydrogen and electron to radiation-induced reactive oxygen and nitrogen species and stabilizes them to less reactive species.[26]

In addition to scavenging the free radicals directly, Q-3-R also has a crucial role in inhibition of enzymes involved in ROS generation such as cyclo-oxygenase, xanthine oxidase, mitochondrial succinoxidase etc.[27] Preadministration of Q-3-R significantly attenuated radiation-induced formation of micronuclei in bone marrow cells of irradiated mice suggesting its potential to protect DNA against radiation damage. In agreement with our report a significant decline in chromosomal aberrations and micronuclei formation was observed by combination of Q-3-R and quercetin in bone marrow cells of 3Gy irradiated mice[12]. Q-3-R has already been reported to exhibit anti-genotoxic potential in irradiated human lymphocytes.[28]

These findings are in congruence with the other studies which have stated the role of antioxidants for minimizing radiation-induced genetic damage.[29,30] Though, the frequency of occurrence of chromosome aberrations, as observed by Samarth and Kumar *et al.* (2003)[30] was not in correlation with our current finding, because a very high lethal dose was used in the current investigation. In our previously published report a combination of Podophyllotoxin and Q-3-R had shown anti-genotoxic potential in mice exposed to lethal dose of radiation[14]. Elaborative studies conducted in our lab had demonstrated that Q-3-R efficiently protected the hematopoietic[24,31] and other radiosensitive organs of mice against radiation damage and supported in their healthy survival.

The present study demonstrates that administration of Q-3-R before radiation exposure could minimize the damage to the genetic material of the irradiated mice. However, the radio-mitigative potential of Q-3-R needs to be investigated, where it may target the pathway of repair after radiation exposure. The other limitation of this study was that only a single dose of Q-3-R was used. Experiments using the multiple dose of Q-3-R can be planned further to investigate its radioprotective effect. Multiple dosing of some antioxidant radioprotectors was associated with their toxicity to the normal tissues.[10] However, the reported toxicity studies revealed that Q-3-R is non-toxic upto 2000 mg/kg.[32] Since this molecule is safe and has been used in clinics for the treatment of osteoarthritis and other neuro-inflammatory diseases[11], it can be further investigated for its radioprotective potential.

5. CONCLUSION

The present study demonstrated that Q-3-R has a potential to reduce radiation-induced chromosomal aberrations, hence minimised the risk of genetic damage when administered prior to radiation exposure. The protective potential of this polyphenol is probably due to its free radical stabilising property. The presence of the phenolic rings in Q-3-R are responsible for antioxidant potential and contribute in hydrogen donation for stabilizing radiation-generated free radicals, hence minimising oxidative stress. Further investigation can be done to prove the efficacy of this bioflavonoid as a modality for other radiation-mediated pathological conditions.

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CONTRIBUTOR

Dr Savita Verma, Sciectist D is presently working in division of Molecular and Radiation Biosciences, Institute of Nuclear Medicine and Allied Sciences(INMAS), Delhi. She is working for the development of a radioprotector from herbal source. Her research interest lies in the area of prevention of radiation-induced toxicity to lungs and hematopoietic system of murine models.