In vitro Protective Effect of Rutin and Quercetin against Radiation-induced Genetic Damage in Human Lymphocytes

Abstract

Purpose of the Study: Rutin (RUT) and quercetin (QRT) which are dietary compounds were investigated for their ability to protect against ionizing radiation (IR)-induced genotoxicity in human lymphocytes. **Materials and Methods:** The radiation antagonistic potential of RUT and QRT was assessed by alkaline comet and cytokinesis-block micronucleus (CBMN) assay. **Results:** Treatment of lymphocytes with RUT and QRT (25 μg/ml) prior exposure to 2 Gy gamma radiation resulted in a significant reduction of frequency of micronuclei as compared to the control set of cells evaluated by CBMN assay. Similarly, treatment of lymphocytes with RUT and QRT before radiation exposure showed significant decrease in the DNA damage as assessed by comet parameters, such as percent tail DNA and olive tail moment. **Conclusion:** The study demonstrates the protective effect of RUT and QRT against IR-induced DNA damage in human lymphocytes, which may be partly attributed to scavenging of IR-induced free radicals and also by the inhibition of IR-induced oxidative stress.

Keywords: *DNA damage, free radicals, micronuclei, quercetin, rutin*

Shrikant L. Patil, K. Swaroop[1], Nilesh Kakde[2], H. M. Somashekarappa[1]

Department of Physiology, K. S. Hegde Medical Academy, Nitte University, [1]Centre for Application of Radioisotopes and Radiation Technology, USIC, Mangalore University, Mangalore, Karnataka, [2]Department of Botany, Shri Vyankatesh Arts, Commerce and Science College, Buldhana, Maharashtra, India

Introduction

Ionizing radiation (IR) is considered ubiquitous environmental carcinogen damaging DNA directly by energy deposition or indirectly by the generation of reactive oxygen species (ROS) and free radicals.[1] ROS and free radicals react with cellular macromolecules (i.e., proteins, carbohydrates, lipids, and nucleic acids) leading to cell death or mutations and chromosome instability when they are not repaired or misrepaired. Several endogenous antioxidant enzymes (such as superoxide dismutase, catalase, and glutathione peroxidase) are capable of scavenging ROS and have shown pivotal role in the repair of DNA damage induced by ROS.[2-5]

The risk of radiation-induced malignancies as a result of genotoxicity to normal cells is one of the most serious problems during radiotherapy in patients with disease-free survival. Tumor control without producing damage to the surrounding normal tissues through the use of chemical agents has a clinical relevance in radiotherapy. Therefore, protection of surrounding normal tissues has a practical relevance in improving the therapeutic outcome. Although many

natural and synthetic/semi-synthetic chemicals have been investigated in the recent past for their efficacy to reduce adverse effects of IR,[6,7] the inherent toxicity of some of the synthetic agents at their radioprotective concentrations necessitated the further search for secured and efficient compounds.[5] In view of these limitations, an approach to evaluate the radiation protective ability of nontoxic and physiologically acceptable compounds seems to be hopeful and deserves investigation. Neutralization of ROS/reactive nitrogen species (RNS) is one such mechanism by which antioxidants influence the indirect action of radiation.[6] Hence, an appropriate antioxidant intercession seems to inhibit or reduce free radical toxicity and thus offer protection against radiation. The uses of medicinal plants in traditional medicine are widespread, and they serve as leads for the development of novel pharmacological agents. A number of dietary antioxidants and medicinal plants have been reported for their hepatoprotective, neuroprotective, anti-inflammatory, and also antioxidant or radical scavenging properties.[2-5] It may be, therefore, logical to expect that extracts and bioactive compounds with an antioxidant potential derived from plants

Address for correspondence:
Dr. Shrikant L. Patil,
Department of Physiology,
K. S. Hegde Medical
Academy, Nitte University,
Mangalore - 575 018,
Karnataka, India.
E-mail: shrikantlpatil@gmail.com

may render radioprotection to normal tissues as evidenced from earlier reports.[7-18]

Flavonoids are polyphenolic compounds containing a unique structure (diphenyl propane structure). Many varieties of flavonoids have been found in herbs, vegetables, fruits, and beverages.[10] Rutin (RUT) and quercetin (QRT) are having anti-inflammatory potential, which has been demonstrated in a number of animal studies.[10-13] In addition, they also exert potent antioxidant activity and Vitamin C-sparing action.[15] In spite of the considerable quantity of work published about flavonoids, little information is available about the cytogenetic evaluation of flavonoids as protector agent on irradiated cells.[10-19]

The purpose of the present investigation is to evaluate the antigenotoxic and antiapoptotic potential of RUT and QRT on the human lymphocytes grown *in vitro* exposed to gamma radiation.

Materials and Methods

Isolation of lymphocytes

In this study, human peripheral blood lymphocytes were obtained by collecting blood from the consented healthy donors. The selection of donor was done according to the current guidelines of the International Programme on Chemical Safety in humans.[20] Peripheral blood (4–5 ml) was collected aseptically by venipuncture from healthy male individuals (mean age 35 years) in heparinized tubes. Lymphocytes were isolated using Ficoll-Paque according to the manufacturer's instructions. The lymphocyte layer was separated by centrifugation at room temperature and washed twice in phosphate-buffered saline at 1000 rpm for 10 min each. Approximately 106 freshly isolated lymphocytes were cultured in 15 ml sterile tubes containing 5 ml of RPMI-1640 supplemented with 15% fetal calf serum, L-glutamine, streptomycin, PHA, and cultures were then incubated at 37°C in humidified 5% CO_2 atmosphere.

Chemicals

The routine chemicals were procured from Qualigens Fine Chemicals (A Division of GlaxoSmithKline Pharmaceuticals Limited), Mumbai, India.

Experimental design

A fixed number (10^6) of lymphocytes were inoculated into several individual culture T-flasks and allowed to grow. A pilot experiment was carried out to select the optimum concentration of RUT and QRT wherein lymphocytes were treated as follows:
1. Untreated control group: The culture of this group was without RUT, QRT, and radiation
2. RUT and QRT alone group: The culture of this group was treated with of RUT and QRT (50 µg/ml) for 1 h before sham-irradiation

3. Radiation (IR) alone group: The culture of this group was exposed to 2 Gy of gamma radiation
4. RUT and QRT + IR group: The cells of this group were treated with different concentrations (5–50 µg/ml) of RUT and QRT for 1 h before exposure to 2 Gy gamma radiation.

The radiation antagonistic potential of optimal RUT and QRT concentration was evaluated treating the lymphocytes as follows.
1. IR alone group: The cultures of this group were exposed to different doses (0–4 Gy) of gamma radiation
2. RUT and QRT + IR group: The cultures in this group were treated with optimum concentration (10 µg/ml) of RUT and QRT for 1 h before exposure to different doses (0–4 Gy) of gamma radiation.

After the various treatments, cells from the above groups were subjected to micronucleus and comet assays.

Cell irradiation procedure

Exponentially growing lymphocytes (70%–80% confluency) were treated with or without RUT and QRT prior exposure to gamma radiation from ^{60}Co gamma teletherapy facility (Theratron Atomic Energy Agency, Canada) at the Shirdi Saibaba Cancer Hospital, Manipal, at a dose rate of 1 Gy/min and source to surface distance of 73 cm. The dosimeteric performances were routinely conducted by the radiation physicists.

Micronucleus assay

The cytokinesis-block micronucleus (CBMN) assay was carried out according to the method of Fenech and Morley[21] After various treatments, lymphocyte cultures were incubated at 37°C and 44 h from the culture initiation, cytochalasin B (5 µg/ml) was added to arrest cytokinesis. The cultures were harvested at 72 h after initiation and cells were collected by centrifugation. These lymphocytes were subjected to a mild hypotonic (0.56% potassium chloride) treatment for 2 min, centrifuged and fixed in Carnoy's fixative (3:1 methanol, acetic acid).

Comet assay (single cell gel electrophoresis) for DNA damage

The comet assay is a widely used genotoxicological technique for measuring DNA damage in individual cells using alkaline gel electrophoresis. On electrophoresis, the negatively charged cellular DNA fragments migrate toward the anode, appear like a comet with tail and are detected with an image analysis system. This assay was performed under alkaline conditions according to the procedure of Singh *et al.*[22] with minor modifications of Collins *et al.*[23]

Statistical analysis

The experimental data were expressed as mean ± standard error of the mean. The significance of the differences between treatments and respective controls was analyzed

using Student's *t*-test and one-way ANOVA with Bonforroni's *post hoc* test using GraphPad Prism 5. (GraphPad Software, Inc. La Jolla, CA 92037 USA).

Results

Antigenotoxic potential of rutin in human lymphocytes

Micronucleus assay

RUT showed protection against the radiation-induced DNA damage, as evidenced by the significant ($P < 0.01$) reduction in micronucleated binucleate cells (MNBNC) after various doses of RUT treatment in human lymphocytes cells. RUT by itself was not toxic to DNA. Pretreatment of human lymphocytes cells with various concentrations of RUT (0–25 µg/ml) for 1 h reduced the radiation-induced micronuclei (MN).

Although, the reduction in the yield of radiation-induced MN was observed in all the doses, RUT at a dose of 25 µg/ml resulted in a maximum inhibition in the yield of MNBNC when compared with other RUT-treated groups. However, increase in the dose of RUT did not further enhance the radioprotective potential. Therefore, 25 µg/ml of RUT was selected as an optimal dose for further studies [Table 1].

Cytokinesis-blocked proliferation index (CBPI) was calculated to assess the effect of RUT on the cytotoxic effect of irradiated human lymphocytes cells. The values of CBPI in IR alone showed delay in cell proliferation. RUT pretreatment modulated the proliferative activity in the combination groups with an increase in CBPI, indicating surmount of cytotoxic effects caused by IR [Table 2].

The optimal dose of RUT, 25 µg/ml with the greatest reduction in MN, was further used in combination with various doses of gamma radiation (0, 1, 2, 3, and 4 Gy) exposed 1 h after RUT treatment to assess the antigenotoxic potential even at the higher doses of radiation [Table 3]. A linear dose-dependent MNBNC% increase in radiation alone group was observed while 25 µg/ml RUT significantly resulted in 26.2%, 25.1%, 29.9%, and 21.2% reduction of MNBNC, when compared to the respective radiation alone groups.

Comet assay

Human lymphocytes cells treated with 2 Gy of radiation caused a significant ($P < 0.01$) DNA damage as evident by the increase in tail DNA and olive tail moment (OTM) when compared with control group.

The optimum protective dose of the RUT was selected by treating human lymphocytes cells with 0, 5, 10, 25 µg/ml RUT for 1 h before exposure to 2 Gy of gamma radiation at 60 min of postirradiation time. Treatment of human lymphocytes cells with different doses of RUT reduced the DNA strand breaks significantly, the highest reduction was observed at 25 µg/ml RUT [Table 4].

Table 1: Effect of rutin on the micronuclei-induction in the human lymphocytes cells exposed to 2 Gy gamma radiation

Group	MNBC/1000 cells±SEM			Total MN
	One	Two	Multiple	
Untreated	12.05±1.21	1.40±0.56	0.0±0.0	12.09±1.33
RUT (25 µg) alone	11.95±1.78	1.45±0.14	0.54±0.03	12.30±1.37
IR alone (2 Gy)	232.26±4.96	25.14±1.23	6.05±0.78	249.05±4.92[a]
RUT (10 µg) + IR	180.51±4.08	12.05±1.89	4.12±1.57	189.24±3.54[b]
RUT (15 µg) + IR	169.28±3.54	9.36±1.47	2.51±1.21	181.82±3.07[c]
RUT (20 µg) + IR	177.62±3.72	9.71±1.47	2.14±1.34	189.25±2.72[c]
RUT (25 µg) + IR	164.71±3.47	8.75±2.12	1.46±0.56	174.65±3.68[c]

All results are shown as mean±SEM from the data of three independent experiments. The significant levels [a]*P*<0.05, [b]*P*<0.01, [c]*P*<0.001 and no symbol: Nonsignificant, when compared with IR alone group. RUT: Rutin, IR: Radiation, SEM: Standard error of mean, MNBC: Micronuclei in buccal cells, MN: Micronucleus

Table 2: Effect of radiation on cytokinesis blocked proliferation index values in the human lymphocytes cells exposed to 2 Gy gamma radiation

RUT (µg/mL)	CBPI	Cytostasis (%)
0	1.60±0.06	42.74
10	1.67±0.07	34.12
15	1.74±0.05	26.20
20	1.72±0.07	20.68
25	1.59±0.05	38.07

All results are shown as mean±SEM from the data of three independent experiments. CBPI: Cytokinesis-blocked proliferation index, RUT: Rutin, SEM: Standard error of mean

Table 3: Frequency of micronuclei and percentage inhibition in human lymphocytes cells treated with 25 µg/mL of rutin before exposure to different doses of gamma radiation

IR dose (Gy)	Total MN/1000 BNC±SEM		MN inhibition (%)
	IR alone	RUT+IR	
0	12.90±1.47	14.090±1.38	-
1	109.24±2.32	81.50±1.91a	26.2
2	171.18±2.78	130.25±2.04b	25.1
3	262.05±3.24	185.75±2.28c	29.9
4	359.50±3.47	285.00±2.68b	21.2

All results are shown as mean±SEM from the data of three independent experiments. The significant levels [a]*P*<0.05, [b]*P*<0.01, [c]*P*<0.001 and no symbol: Nonsignificant, when compared with respective radiation alone group. IR: Radiation, SEM: Standard error of mean, MN: Micronuclei, BNC: Binucleated cells, RUT: Rutin

A separate experiment was conducted to study the influence of RUT on the DNA damage induced by different doses of gamma radiation. Treatment of lymphocytes with different

doses of gamma radiation caused an elevation in the DNA damage in a linear dose-dependent manner ($r^2 = 0.9870$) measured as percent tail DNA and mean OTM. Treatment with optimal dose of RUT (25 µg/ml) for 1 h before exposure with different doses of radiation caused a significant reduction ($P < 0.05$) in the percentage of tail DNA and OTM values.

Effect of quercetin on radiation induced micronuclei

The frequencies of MN in human lymphocytes induced by QRT or in combination with 2 Gy radiation are shown in Table 5. No significant increase in the number of MN and micronucleated cells observed with QRT alone (25 µg/ml) as compared to the untreated group. After various concentrations of QRT treatment, there was no inhibition of lymphocyte divisions as the number of binucleated cells (BNC) and CBPI values were not significantly different from the control. Pretreatment of lymphocytes with various concentration of QRT 1 h before 2 Gy gamma radiation significantly reduced the frequency of MN as compared to the control cells which were irradiated without QRT [Table 5]. Among all the doses of QRT screened, 25 µg/ml showed the highest reduction in MN when compared with other QRT pretreated groups. Therefore, this concentration of QRT was considered as an optimal protective concentration and further experiments were carried out.

The frequency of MNBNC with one, two and multiple MN is presented in Table 5. In general, the frequency of BNCs bearing MN increased in a linear dose-dependent manner ($r^2 = 0.999$) and the highest number of MN was observed at 4 Gy. Pretreatment of lymphocytes with 25 µg/ml QRT significantly ($P < 0.01$) reduced the MN in a linear dose-dependent manner ($r^2 = 0.985$) when compared to the respective radiation alone group.

The treatment with QRT alone did not influence cell division, the number of BNCs and CBPI values were not different from those found in the control [Table 6]. QRT pretreatment with irradiation resulted in a significant decline in the induction of cells with one, two, and multiple MN. The frequencies of two and multiple MNBNC were always remained lower in QRT pretreated group [Table 6].

Effect of quercetin on radiation induced DNA damage

The results of the genotoxicity studies by comet assay are shown in Table 7, QRT at 25 µg/ml concentration did not induce any significant alteration of the comet parameters as compared with that from unirradiated control cells. Data from Table 7 shows increase in comet parameters, such as percent DNA in the tail and mean OTM of lymphocytes which were exposed to 2 Gy of gamma radiation. These results further revealed that the presence of QRT (10–25 µg/ml) progressively decreased the comet assay parameters in irradiated lymphocytes. However, the

Table 4: Induction of DNA damage assessed by alkaline comet assay (percentage tail DNA and olive tail moment) in human lymphocytes treated with different concentrations of rutin for 1 h before exposure to 2 Gy of gamma radiation

Group	Comet parameters	
	Percentage tail DNA±SEM	OTM±SEM
Untreated	6.07±0.21	1.58±0.27
RUT (25 µg) alone	6.95±0.42	4.05±0.24
IR alone (2Gy)	26.32±1.97	25.16±2.67
RUT (10 µg) + IR	17.44±0.95[a]	17.12±1.25[a]
RUT (15 µg) + IR	15.56±1.82[a]	17.54±1.98
RUT (20 µg) + IR	15.46±2.69[c]	16.87±1.72[c]
RUT (25 µg) + IR	13.92±1.85[b]	15.77±1.36[b]

All results are shown as mean±SEM from the data of three independent experiments. The significant levels [a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$ and no symbol: Nonsignificant, when compared with radiation alone group. OTM: Olive tail moment, IR: Radiation, RUT: Rutin, SEM: Standard error of mean

Table 5: Induction of micronuclei and cytokinesis blocked proliferation index values in human lymphocytes treated with different concentrations of quercetin (for 1 h) alone or exposed with 2 Gy of gamma radiation

Group	MNBC/1000 cells±SEM			Total MN
	One	Two	Multiple	
Untreated	15.34±0.51	1.72±0.35	0.0±0.0	17.06±1.45
QRT (25 µg) alone	16.06±1.48	1.45±0.47	0.50±0.29	18.71±1.65
IR alone (2Gy)	154.26±5.47	11.24±1.11	6.25±1.08	178.25±8.84[a]
QRT (10 µg) + IR	143.62±3.72	9.81±1.47	6.14±1.34	159.25±2.72[c]
QRT (15 µg) + IR	138.28±3.54	7.36±1.47	4.51±1.21	147.82±3.07[c]
QRT (20 µg) + IR	136.85±3.05	8.45±1.89	3.42±1.76	155.24±3.54[b]
QRT (25 µg) + IR	132.71±3.47	6.75±2.12	2.46±0.56	142.65±3.68[c]

Group	Percentage MN reduction	CBPI
Untreated	-	1.962
QRT (25 µg) alone	-	1.952
IR alone (2Gy)	-	1.729
QRT (10 µg) + IR	15.15	1.632
QRT (15 µg) + IR	27.80	1.655
QRT (20 µg) + IR	17.90	1.642
QRT (25 µg) + IR	9.10	1.597

All results were shown as mean±SEM from the data of three independent experiments. The significant levels [a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$ and no symbol: Nonsignificant, when compared with IR alone group. CBPI: Cytokinesis blocked proliferation index, QRT: Quercetin, IR: Radiation, MNBC: Micronuclei in buccal cells, MN: Micronucleus, SEM: Standard error of mean

effect was not dose dependent; 25 µg/ml showed better effect than other doses.

Table 6: Induction of micronuclei and cytokinesis blocked proliferation index values in human lymphocytes treated with optimum concentration of quercetin (25 μg/mL) for 1 h before exposure to different doses of gamma radiation

IR dose (Gy)	MNBC/1000 cells±SEM			Total MN	CBPI
	One	Two	Multiple		
0	17.01±0.71	1.14±0.47	0.78±0.15	19.10±1.56	1.954
1	52.06±1.48	4.92±1.26	0.45±0.47	59.30±0.29[a]	1.827
2	117.96±4.47	3.39±1.07	3.64±1.31	132.15±4.08[a]	1.765
3	137.15±3.05	18.90±2.65	12.05±1.52	171.32±6.76[b]	1.652
4	205.98±3.54	27.07±2.82	16.06±1.47	239.60±1.21[b]	1.557

All results were shown as mean±SEM from the data of three independent experiments. The significant levels [a]P<0.01, [b]P<0.001, when compared with respective radition alone group. CBPI: Cytokinesis blocked proliferation index, IR: Radiation, MNBC: Micronuclei in buccal cells, MN: Micronucleus, SEM: Standard error of mean

Table 7: Induction of DNA damage assessed by alkaline comet assay (percentage tail DNA and olive tail moment) in human lymphocytes treated with different concentrations of quercetin for 1 h before exposure to 2 Gy of gamma radiation

Group	Comet parameters	
	Percentage tail DNA±SEM	OTM±SEM
Untreated	7.07±0.51	1.72±0.35
QRT (25 μg) alone	7.45±0.48	3.85±0.47
IR alone (2Gy)	25.26±2.47	24.24±2.11
QRT (10 μg) + IR	19.71±1.47[a]	17.75±2.12
QRT (15 μg) + IR	16.95±1.05[a]	16.65±1.89[a]
QRT (20 μg) + IR	15.62±3.72[c]	16.22±1.47[c]
QRT (25 μg) + IR	14.28±1.54[b]	15.36±1.47[b]

All results are shown as mean±SEM from the data of three independent experiments. The significant levels [a]P<0.05, [b]P<0.01, [c]P<0.001 and no symbol: Nonsignificant, when compared with radiation alone group. OTM: Olive tail moment, QRT: Quercetin, IR: Radiation, SEM: Standard error of mean

A separate experiment was conducted to study the influence of QRT on the DNA damage induced by different doses of gamma radiation. Treatment of lymphocytes with different doses of gamma radiation caused elevation in the DNA damage in a linear dose dependent manner (r^2 = 0.9870) measured as percent tail DNA and mean OTM. Treatment with 25 μg/ml QRT for 1 h before exposure to different doses of gamma radiation demonstrated a dose-dependent reduction in the DNA damage in QRT + IR group when compared with irradiation alone treatment. The reduction in the DNA damage by QRT was statistically significant at all doses of IR, when compared with IR-treatment alone.

Discussion

IR is a physical agent known to induce mutations, chromosomal aberrations and considered as a Group I potential human carcinogen by the International Agency for Research on Cancer.[1-3] However, IR has gained great

importance in the treatment of different types of cancers. One of the biological effects of IR is the generation of ROS, some of which can induce a variety of DNA lesions[4-6] by both direct energy deposition on DNA (direct effect) and reactions with diffusible water radicals leading to generation of hydroxyl radicals (indirect effect)[6] which subsequently results in DNA damage (mutation) or cell death.[7] Therefore, the study of cell and tissue responses to IR is an important task in both radiation biology and oncology. Regarding the side effects caused by IR in patients undergoing radiotherapy, the radioprotectors undeniably have an important role for tolerance and increasing the survival rate in patients.[6] The potential applications of radio protective substances include their use in the event of radiation accident or in radiation therapy of cancer patients to protect normal cells. Several natural products have been shown to protect cells against radiation damage by virtue of their antioxidant properties.[4-9]

In the present study, we evaluated the efficacy of RUT and QRT in reducing effects of gamma radiation. RUT and QRT, a phenolic antioxidants, play an important role in the oxidation process by being preferentially oxidized by the attacking radical, due to high redox potential, it can act as reducing agents, hydrogen donors, singlet oxygen quenchers, and as metal chelating agents, giving intrinsic antioxidant properties.[10-15] To the best of our knowledge, no study has been conducted earlier to determine the antigenotoxic and radioprotective effects of RUT and QRT in human lymphocytes. In the present study, the gamma radiation damage on human lymphocytes was studied employing MN, comet, and apoptosis assays, each of which has its own importance in drawing inference.

IR, as many other cytotoxic agents, may induce whole chromosomes or chromosome fragments that do not attach to the spindle apparatus during mitosis. These chromosomes or fragments then may be spontaneously enclosed by nuclear membrane, forming a micronucleus. According to CBMN assay, our results clearly indicated the nontoxic nature of RUT and QRT by itself. They caused a moderate decrease in CBPI with decline in total number of MN in gamma irradiated samples at all the tested concentrations when compared with IR alone group indicating its antigenotoxic potential. Our results are in agreement with reports from previous genotoxicity studies where treatment of human lymphocytes with WR-2721 (7 mM) before exposure to 2 Gy gamma radiation significantly reduced radiation induced MN formation,[16,17] similar radioprotective effect was also observed with other well-known antioxidants.[16-19]

In the present study, a significant radioprotective effect was observed as indicated by the reduced genotoxicity at an optimal dose of 25 μg/ml, RUT and QRT with no further benefit with its higher concentrations. Genotoxic effects of IR are also mediated through formation of free radicals or ROS/RNS, which additionally cause DNA single-strand or

double-strand breaks, alkali-labile, and in excision repair sites.[24-26] All the above-mentioned lesions significantly contribute to the increased levels of primary DNA damage that could be detected by the alkaline comet assay.

The antigenotoxic potential of RUT and QRT were also further confirmed using alkaline single-cell electrophoresis assay (comet assay). This assay is highly sensitive, single-cell-based, rapid, and reproducible, needs only a small number of cells and therefore used extensively for the evaluation of DNA damage and repair studies.[18-20] As the DNA damage produced is directly related to the radiation dose, any change in radiation dose should be revealed in a proportional change in the comet measurement. Thus, the size and shape of the comet and the distribution of DNA within the comet have been correlated with the extent of DNA damage.[18] The results of present alkaline comet study indicate that RUT and QRT without being toxic to cells at the concentrations used, have the ability to reduce the comet parameters such as tail DNA and OTM which were induced in lymphocytes following various doses of gamma rays. These results are in agreement with observation of earlier author that amifostine decreased radiation-induced DNA damage in lymphocytes assessed by comet assay.[22] So far, several antioxidants and free radical scavengers have been found to attenuate IR-induced apoptosis and exhibited radioprotection.[10-15]

This is in agreement with our findings in the present study. We observed apoptotic morphological changes and DNA fragmentation in irradiated lymphocytes. Pretreatment of lymphocytes with RUT and QRT alone was found to be nontoxic at the concentration used and in combination with IR showed a significant suppression of morphological changes with a reduction in MN induction and DNA damage. These findings are in agreement with earlier observations for hesperidin and cimetidine in cultured human peripheral blood lymphocytes exposed to irradiation.[26-30] The studies are in progress to elucidate the exact mechanism of antigenotoxic effect of RUT and QRT in human lymphocytes.

Conclusion

To conclude, the antigenotoxic effects rendered by the RUT and QRT to mitigate the radiation induced damage in human lymphocytes may be partly attributed to scavenging of radiation induced ROS and also by the inhibition of radiation induced oxidative stress.

Conflicts of interest

There are no conflicts of interest.

References

1. Hall EJ, Giaccia AJ. Radiobiology for the Radiologist. 6th ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2006.
2. Devasagayam TP, Sainis KB. Immune system and antioxidants, especially those derived from Indian medicinal plants. Indian J Exp Biol 2002;40:639-55.
3. Mantena SK, Mutalik S, Srinivasa H, Subramanian GS, Prabhakar KR, Reddy KR, *et al.* Antiallergic, antipyretic, hypoglycemic and hepatoprotective effects of aqueous extract of *Coronopus didymus* Linn. Biol Pharm Bull 2005;28:468-72.
4. Nair CK, Parida DK, Nomura T. Radioprotectors in radiotherapy. J Radiat Res 2001;42:21-37.
5. Uma Devi P. Radioprotective, anticarcinogenic and antioxidant properties of the Indian holy basil, *Ocimum sanctum* (Tulasi). Indian J Exp Biol 2001;39:185-90.
6. Hosseinimehr SJ, Ahmadi A, Mahmoudzadeh A, Mohamadifar S. Radioprotective effects of daflon against genotoxicity induced by gamma irradiation in human cultured lymphocytes. Environ Mol Mutagen 2009;50:749-52.
7. Arora R, Chawla R, Puri SC, Sagar R, Singh S, Kumar R, *et al.* Radioprotective and antioxidant properties of low-altitude *Podophyllum hexandrum* (LAPH). J Environ Pathol Toxicol Oncol 2005;24:299-314.
8. Maurya DK, Devasagayam TP, Nair CK. Some novel approaches for radioprotection and the beneficial effect of natural products. Indian J Exp Biol 2006;44:93-114.
9. Chen N, Zhao SJ. Radioprotection related activities of medicinal plant. Zhongguo Zhong Yao Za Zhi 2007;32:1263-6.
10. Patil SL, Mallaiah SH, Patil RK. Antioxidative and radioprotective potential of rutin and quercetin in Swiss albino mice exposed to gamma radiation. J Med Phys 2013;38:87-92.
11. Patil SL, Somashekarappa H, Rajashekar K. Radiomodulatory role of rutin and quercetin in swiss albino mice exposed to the whole body gamma radiation. Indian J Nucl Med 2012;27:237-42.
12. Patil SL, Somashekarappa HM, Patil R. Evaluation of the radioprotective action of rutin in mice exposed to gamma-radiation. Int J Biol Pharm Res 2012;3:12-8.
13. Patil SL, Somashekarappa HM, Patil R. Ameliorative effect of rutin against oxidative stress in mice induced by gamma-irradiation. Res J Pharm Biol Chem Sci 2011;2:694-701.
14. Patil SL, Somashekarappa HM, Patil R. Radioprotective Effect of Quercetin in Swiss Mice. Vol. 2. Proceedings of NAARRI International Conference; 2010. p. 45-50.
15. Patil SL, Rao NB, Somashekarappa HM, Rajashekar KP. Antigenotoxic potential of rutin and quercetin in swiss mice exposed to gamma radiation. Biomed J 2014;37:305-13.
16. Vrinda B, Uma Devi P. Radiation protection of human lymphocyte chromosomes *in vitro* by orientin and vicenin. Mutat Res 2001;498:39-46.

17. Hosseinimehr SJ, Mahmoudzadeh A, Ahmadi A, Mohamadifar S, Akhlaghpoor S. Radioprotective effects of hesperidin against genotoxicity induced by gamma-irradiation in human lymphocytes. Mutagenesis 2009;24:233-5.

18. Olive PL, Banáth JP. The comet assay: A method to measure DNA damage in individual cells. Nat Protoc 2006;1:23-9.

19. Archana PR, Nageshwar Rao B, Ballal M, Satish Rao BS. Thymol, a naturally occurring monocyclic dietary phenolic compound protects Chinese hamster lung fibroblasts from radiation-induced cytotoxicity. Mutat Res 2009;680:70-7.

20. Albertini RJ, Anderson D, Douglas GR, Hagmar L, Hemminki K, Merlo F, *et al*. IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. International programme on chemical safety. Mutat Res 2000;463:111-72.

21. Fenech M, Morley AA. Measurement of micronuclei in lymphocytes. Mutat Res 1985;147:29-36.

22. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 1988;175:184-91.

23. Collins A, Dusinská M, Franklin M, Somorovská M, Petrovská H, Duthie S, *et al*. Comet assay in human biomonitoring studies: Reliability, validation, and applications. Environ Mol Mutagen 1997;30:139-4.

24. Prabhakar KR, Veerapur VP, Parihar KV, Priyadarsini KI, Rao BS, Unnikrishnan MK. Evaluation and optimization of radioprotective activity of *Coronopus didymus* Linn. In gamma-irradiated mice. Int J Radiat Biol 2006;82:525-36.

25. Rao BS, Shanbhoge R, Upadhya D, Jagetia GC, Adiga SK, Kumar P, *et al*. Antioxidant, anticlastogenic and radioprotective effect of coleus aromaticus on Chinese hamster fibroblast cells (V79) exposed to gamma radiation. Mutagenesis 2006;21:237-42.

26. Rao BS, Upadhya D, Adiga SK. Protection of ionizing radiation-induced cytogenetic damage by hydroalcoholic extract of *Cynodon dactylon* in Chinese hamster lung fibroblast cells and human peripheral blood lymphocytes. J Environ Pathol Toxicol Oncol 2008;27:101-12.

27. Müller AC, Pigorsch S, Beyer C, Lautenschläger C, Dunst J. Radioprotective effects of amifostine *in vitro* and *in vivo* measured with the comet assay. Strahlenther Onkol 2004;180:517-25.

28. Yoshida A, Pommier Y, Ueda T. Endonuclease activation and chromosomal DNA fragmentation during apoptosis in leukemia cells. Int J Hematol 2006;84:31-7.

29. Kojima Y, Kondo T, Zhao QL, Shoji M, Futatsuya R. Protective effects of cimetidine on radiation-induced micronuclei and apoptosis in human peripheral blood lymphocytes. Free Radic Res 2002;36:255-63.

30. Kalpana KB, Devipriya N, Srinivasan M, Menon VP. Investigation of the radioprotective efficacy of hesperidin against gamma-radiation induced cellular damage in cultured human peripheral blood lymphocytes. Mutat Res 2009;676:54-61.