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Quercetin as a radiosensitizer for enhanced efficacy of radiotherapy in MCF-7 breast cancer cells

Mohammad Banparvar [1], Hamid Saeidi Saedi [2], Mona Haddad Zahmatkesh [3] *

[1] Student Research Committee, School of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran

[2] Department of Radiation Oncology, GI Cancer Screening and Prevention Research Center, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran

[3] Department of Pharmaceutical Biotechnology and Nuclear Pharmacy, School of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran

Abstract

Introduction: Radiation therapy is a primary approach for treating cancer. Utilizing natural radiosensitizer compounds is crucial to enhance radiosensitivity in tumor tissue while minimizing damage to normal tissue. This study aims to assess the impact of quercetin as a radiosensitizing compound in MCF-7 cells.

Materials and Methods: This research examined the impact of quercetin at concentrations of 20, 40, and 60 µM with and without radiation (2 and 3 Gy) on the MCF-7 breast cancer cell line as a radiosensitizer agent. The investigation employed a micronucleus test, clonogenic assay, and assessments of Superoxide-dismutase and catalase activity.

Results: The findings indicated that the group exposed to radiation exhibited a significant decrease in the number of colonies ($P < 0.0001$) and activity of SOD and CAT enzymes while showing a significant increase in the number of micronuclei compared to the control group ($P < 0.0001$). Additionally, in all the groups treated with quercetin and exposed to radiation, there was a notable increase in micronuclei and a significant decrease in the number of colonies and activity of CAT and SOD enzymes.

Conclusions: The study's findings demonstrated that quercetin has the ability to increase the sensitivity of MCF-7 breast cancer cells to ionizing radiation in a manner that depends on the dosage.

Keywords: Breast cancer, MCF-7, Radiosensitizing, Radiotherapy

Corresponding Authors: Mona Haddad Zahmatkesh

✉ Email: haddadmona629@gmail.com

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Introduction

Cancer is a medical condition where abnormal cells grow uncontrollably and can spread to other parts of the body through metastasis(1). Breast cancer is a major concern, causing a significant number of cancer-related fatalities in women globally (2). According to the World Health Organization (WHO), cancer is one of the primary causes of death, and in 2020 alone, approximately 9.9 million people passed away due to this condition. Out of these, 2.3 million individuals, which make up 11.7% of the total new cases, passed away due to breast cancer, the second most commonly diagnosed type of cancer (3). Breast cancer is commonly treated with conventional methods such as surgery, hormone therapy, radiotherapy, and chemotherapy (4). In advanced stages of cancer, these treatments rarely work and often cause damage to healthy cells (5). Understanding the key factors and molecular mechanisms of breast cancer metastasis is crucial, as it has a high risk of relapse and can spread to vital organs like the lungs, brain, liver, and bone, leading to fatal outcomes (6). Breast cancer prognosis has improved due to the continuous development of medical technology. However, recurrence and metastasis still pose major challenges (7). Moreover, drug resistance is a common occurrence due to the high variability and compensatory adaptation mechanisms of cancer cells, which may lead to treatment failure. Therefore, it is crucial to develop new therapeutic strategies and drugs to treat breast cancer effectively (8).

Radiotherapy (RT) is a common treatment for cancer that generates reactive oxygen species in tumor tissues, promoting apoptosis and inhibiting tumor growth (9). However, healthy tissues are unavoidably exposed to radiation, increasing the risk of normal tissue complications (10). To increase radiotherapy's efficacy, radiosensitizers are used to absorb and deposit X-ray irradiation energy in tumors (11). Studies suggest that the use of radiosensitizing agents can improve radiotherapy treatment outcomes, leading to better survival rates in patients with breast cancer (10). Quercetin (QUR), a natural compound, has been extensively researched as a radiosensitizer for tumor radiotherapy, demonstrating significant increases in tumor radiosensitivity both in vitro and in vivo. When used systemically, quercetin is considered a radioprotective agent (12).

Quercetin is a flavonoid that is commonly found in many vegetables, fruits, and seeds, such as apples, cherries, grapes, onions, broccoli, peanuts, and soybeans, as well as beverages like tea and wine (13). Researchers have observed that it has anticancer effects, including inhibiting cancer cell growth, invasion, and metastasis, along with regulating autophagy, apoptosis, and immune response enhancement (14). Studies have also shown that quercetin can induce apoptosis and cell cycle arrest in different cancer cell lines, such as breast, prostate, lung, and colon cancers (15). QUR is considered a promising anticancer option because of its chemoprotective action against tumor cell lines through metastasis and apoptosis (16). It influences the G1 phase and induces apoptosis by suppressing cyclin D1, P21, and Twist expression in MCF-7 cells. QUR also plays an anti-proliferative role in MCF-7 cells by reducing the phosphorylation of P38MAPK, a hallmark of cell proliferation (17).

Based on the discovered anticancer effects of flavonoids, including quercetin, it is hypothesized that this compound may affect breast cancer cells and their resistance to radiation therapy, both by reducing and increasing their sensitivity. Therefore, the present study was designed to investigate the radiation sensitization effect of quercetin on MCF-7 breast cancer cells.

Materials and methods

Chemical, Drug, and Reagent

Quercetin and Cytochalasin-B were purchased from Sigma Chemicals Co. (St. Louis, USA). SOD Assay Kit (Nasdox™–Superoxide Dismutase Assay Kit) and CAT kit (Nactaz™ - Catalase Activity Assay Kit) were obtained from Navand Salamat Co. (Iran). Methanol, Giemsa stain, and acetic acid were obtained from Merck (Germany).

Cell line and cell culture

Human breast cancer cell line MCF-7 (obtained from National Cell Bank of Tehran, Iran) was grown in RPMI 1640 medium (Dacell, Iran) supplemented with 10% fetal bovine serum (FBS) (Gibco), 100 units of

penicillin/ml (Dacell, Iran), and 100 µg of streptomycin/ml (Dacell, Iran), incubated at 37 C in 5% CO2. The growth medium was changed every three days and once the cells reached 80% confluence, they were sub-cultured with 0.25% trypsin. (Gibco, UK). (18, 19).

Quercetin treatment and ionizing radiation (IR)

At 24 h after plating the cells, the medium was removed and replaced with a fresh medium or medium containing different concentrations of quercetin. For treatments, cells were left untreated or treated with ionization radiation alone or quercetin in concentrations of 20, 40, and 60 µM in 12-well plates. For drug treatment, quercetin was added to the cultures 4 h before radiation. The control groups were cultured without drug, with corresponding medium amounts instead. The cells that received treatment were subjected to doses of 2 and 3 Gy of IR. The cells were irradiated with a 6 MV X-ray beam produced by a Linear accelerator (Shinva, China) at a dose rate of 1.96 Gy/min and source-to-sample distance (SSD) of 60 cm. Following irradiation, the plates were transferred to the incubator at 37 ◦C under 5% CO2 and 95% humidity.

The cytokinesis-block micronucleus (CBMN) assay

Following the irradiation of MCF-7 cells, the culture medium that contained quercetin was removed and replaced with a fresh medium. The plates were then placed in a CO2 incubator at 37 degrees Celsius and 95% humidity for 48 hours. "To stop proliferation, 100 µl of cytochalasin B with a concentration of 6 µg/ml was added to each well. The cell contents were transferred to microtubes with a 2 ml volume and a fixing solution (6:1 cold glacial acetic acid-methanol solution) was slowly added drop by drop. Each microtube was used to prepare three slides that were left to dry at room temperature for 24 hours before being stained with 10% Gimsa dye for 3 minutes. The slides were washed with distilled water for 30 seconds and dried at room temperature. Finally, the slides were examined under a microscope (20, 21).

Clonogenic assay

The MCF7 cancer cells were seeded in triplicate in 6-well plates at a density of 2000 cells per well. Following an overnight incubation, the cells were subjected to pre-determined groups and treated with or without quercetin for 3 hours. The cells were then exposed to radiation doses of 2 and 3 Gy. The cells were incubated at 37°C in 5% CO2 and 95% humidity for 14 days. Afterward, colonies were washed with PBS, fixed with fixative solution (methanol-acetic acid, 6:1), and stained with 10% Giemsa (v/v) in water. Viable cells were identified based on the presence of colonies with 50 or more cells. The plating efficiency was calculated as follows: PE = (Number of colonies formed / Number of cells plated) × 100%. This allows us to quantify the ability of the cells to form colonies after treatment. The surviving fraction (SF) was determined by dividing the number of colonies formed by the product of the number of cells plated and the plating efficiency(22).

Superoxide dismutase activity assay

To evaluate Superoxide Dismutase (SOD), a Nasdox™eSuperoxide Dismutase Assay Kit (Navand Salamat Company, Urmia, Iran) was used. After preparing a culture medium containing at least one million cells, it was centrifuged at 800 rpm for two minutes, and the supernatant was removed. Then, 500 µl of lysing buffer solution was added to the cells and vortexed for 10 minutes while keeping it on ice. The mixture was centrifuged at a speed of 12000 rpm for 5 minutes. The SOD activity, which is considered an inhibition activity, was determined by measuring the reduction in color development at 405 nm (23).

Catalase activity assay

A commercial kit Catalase (Nactaz™ Catalase Activity Assay Kit, Navand Salamat Company, Urmia, Iran) was utilized to determine catalase (CAT) activity. 1 ml of lysing buffer solution was used to homogenize at least 10^6 cells which were then centrifuged at 8000 rpm for 10 min. The resulting supernatant solution was used as the sample. Following 10 min of incubation at room temperature, catalase was determined by absorbance rate at 550 nm (24).

Statistical analysis

The results were analyzed using GraphPad Prism software (version 7) with Two Way ANOVA - Repeated Measure. Mean ± Standard Deviation (SD) was used to present the data, and any differences with

values of $p \leq 0.05$ were considered statistically significant.

Results

Micronucleus frequency in MCF-7 cells treated with Quercetin and radiation

The micronucleus assay was used to assess genetic damage in MCF-7 cells treated with quercetin and exposed to radiation. The results, illustrated in Figure 1, demonstrate a significant increase in micronucleus frequency at quercetin concentrations of 40 μM and 60 μM, as compared to the control group.

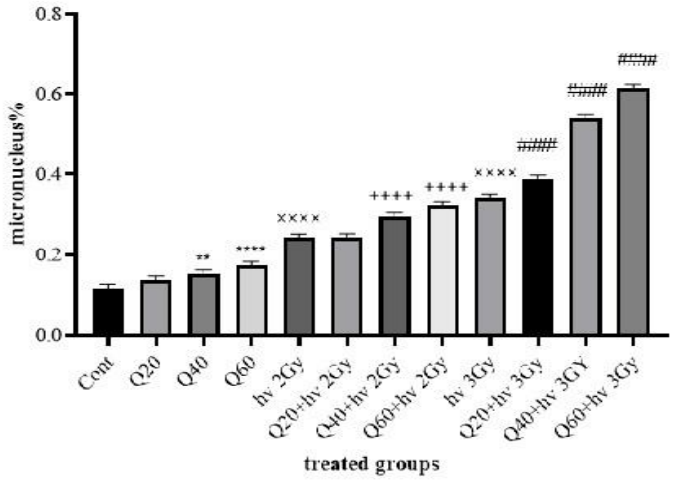

Figure 1. The number of micronuclei in cells treated with radiation and quercetin. (Cont: Count; Q: Quercetin; Gy: Gray; ××××: significant difference with the control group ($P < 0.0001$); **: The significant difference with the control group ($P < 0.01$); ****: significant difference with the control group ($P < 0.0001$); ++++: the significant difference with the group receiving 2 Gy radiation ($P < 0.0001$); ####: the significant difference with the group receiving 3 Gy radiation ($P < 0.0001$).

At a 40 μM concentration, the micronucleus frequency was significantly higher than the control ($P < 0.01$), and at 60 μM, the difference was even more pronounced ($P < 0.0001$). For the groups receiving radiation, the percentage of micronuclei was 0.243% ± 0.01 for the 2 Gy radiation dose and 0.340% ± 0.01 for the 3 Gy dose, compared to 0.015% ± 0.005 in the non-irradiated control.

When quercetin was combined with radiation, the micronucleus frequency increased significantly. Cells treated with 40 μM and 60 μM quercetin along with 2 Gy radiation showed a marked increase in micronuclei ($P < 0.0001$) compared to cells receiving only 2 Gy radiation. Similar effects were observed with the 3 Gy radiation dose. In this case, the percentage of micronuclei in cells treated with quercetin and 3 Gy radiation was 0.388% ± 0.01 (20 μM), 0.539% ± 0.03 (40 μM), and 0.613% ± 0.07 (60 μM), compared to 0.340% ± 0.01 in cells exposed only to 3 Gy radiation.

Clonogenic assay and cell survival fraction in MCF-7 cells treated with Quercetin and radiation

The clonogenic assay demonstrated that ionizing radiation induced cytotoxicity and reduced cell growth (Figure 2). Both 2 Gy and 3 Gy radiation doses resulted in a significant reduction in colony formation compared to the control group ($P < 0.0001$). In the quercetin-treated groups, colony formation was also significantly reduced, with cells treated with 40 μM and 60 μM quercetin showing a significant decrease in the number of colonies compared to the control ($P < 0.0001$).

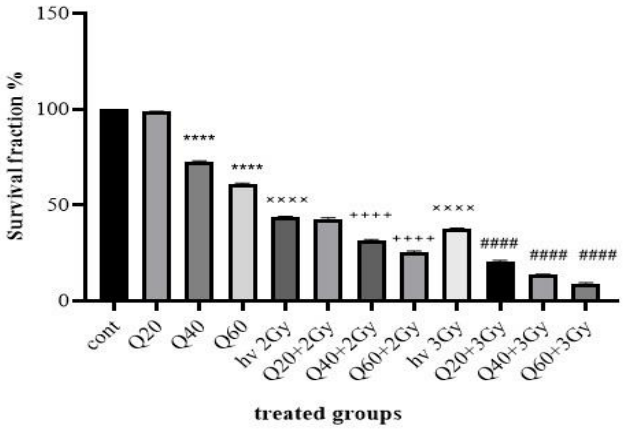

Figure 2. The number of colonies in groups receiving quercetin, radiation, and groups without radiation (Cont: Count; Q: Quercetin; Gy: Gray; ××××: significant difference with the control group ($P < 0.0001$); ****: The significant difference with the control group ($P < 0.0001$); ++++: the significant difference with the group receiving 2 Gy radiation ($P < 0.0001$); ####: the significant difference with the group receiving 3 Gy radiation ($P < 0.0001$)

When quercetin was combined with radiation, a further reduction in colony number was observed. For cells treated with 40 μM and 60 μM quercetin along with 2 Gy radiation, colony formation was significantly lower than in the group exposed only to 2 Gy radiation ($P <$

0.0001). Similarly, for cells treated with 20 μM, 40 μM, and 60 μM quercetin in combination with 3 Gy radiation, colony numbers were significantly reduced compared to the group receiving only 3 Gy radiation (P < 0.0001).

In Vitro measurement of SOD activity level in MCF-7 cell treated with Quercetin and radiation

Superoxide dismutase (SOD) activity was measured in MCF-7 breast cancer cells treated with quercetin and exposed to various radiation doses (Figure 3). The results showed significant differences in SOD activity between treated groups and controls.

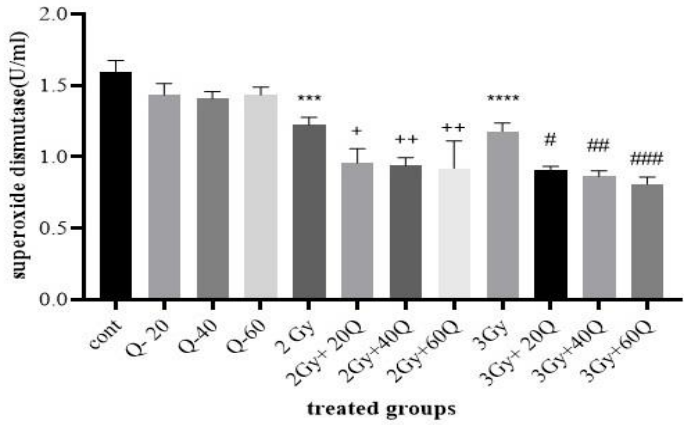

Figure 3. The results of superoxide dismutase enzyme were measured in groups that received quercetin, radiation, and those that did not receive radiation. (Cont: Count; Q: Quercetin; Gy: Gray; ****: significant difference with the control group (P < 0.0001); ++: the significant difference with the group receiving 2 Gy radiation (P < 0.0001); ###: the significant difference with the group receiving 3 Gy radiation (P < 0.0001); ####: the significant difference with the group receiving 3 Gy radiation (P < 0.0001).

At quercetin concentrations of 20, 40, and 60 μM, no significant differences in SOD activity were observed compared to the control group. However, when cells were treated with 40 μM and 60 μM quercetin and exposed to 2 Gy radiation, SOD activity was significantly higher than in the group exposed to 2 Gy radiation alone (P < 0.0001). In contrast, the 20 μM quercetin-treated group exposed to 2 Gy radiation had significantly higher SOD activity compared to the radiation-only group (P < 0.05).

For cells exposed to 3 Gy radiation, SOD activity was significantly different from the control group (P < 0.0001). Furthermore, the groups treated with 40 μM and 60 μM quercetin followed by 3 Gy radiation exhibited significantly higher SOD activity than the 3 Gy-only group (P < 0.001). A significant difference was also observed between the 3 Gy radiation group and the 20 μM quercetin-treated group (P < 0.05).

In Vitro measurement of CAT activity level in MCF-7 cell treated with Quercetin and radiation

Catalase (CAT) activity was measured in MCF-7 cells treated with quercetin and exposed to radiation. Figure 4 illustrates the enzyme activity levels across the different experimental groups.

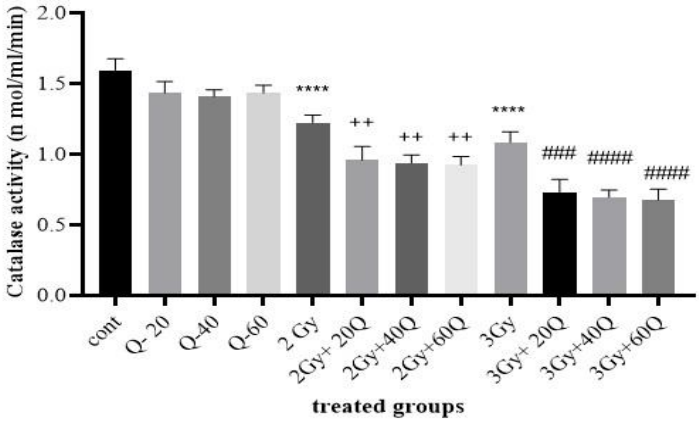

Figure 4. The result of catalase enzyme in groups receiving quercetin, radiation, and groups without radiation. (Cont: Count; Q: Quercetin; Gy: Gray; ***: significant difference with the control group (P < 0.001); ****: The significant difference with the control group; +: the significant difference with the group receiving 2 Gy radiation (P < 0.05); ++: the significant difference with the group receiving 2 Gy radiation (P < 0.0001); #: the significant difference with the group receiving 3 Gy radiation (P < 0.05);); ##: the significant difference with the group receiving 3 Gy radiation (P < 0.01);####: the significant difference with the group receiving 3 Gy radiation (P < 0.0001).

No significant differences in catalase activity were observed between the quercetin-treated groups and the control group. However, in the radiation-only groups (2 Gy and 3 Gy), catalase activity was significantly decreased compared to the control group (P < 0.0001).

When quercetin was combined with radiation, a significant increase in CAT activity was observed. Cells treated with 20, 40, and 60 μM quercetin along with 2 Gy radiation showed significantly higher catalase activity compared to the 2 Gy-only group (P <

0.01). Additionally, cells treated with quercetin (20, 40, or 60 μM) and exposed to 3 Gy radiation exhibited significantly lower CAT activity compared to the 3 Gy-only group ($P < 0.0001$). Specifically, catalase activity in these groups was reduced to 0.731 ± 0.08, 0.695 ± 0.05, and 0.675 ± 0.07, respectively, compared to 1.083 ± 0.07 in the 3 Gy radiation-only group.

Discussion

Breast cancer is still the most commonly diagnosed cancer in women. While there have been fewer cases diagnosed in advanced, metastatic stages in recent decades, it remains a significant public health concern globally (25). The typical treatments for breast cancer include surgery, radiotherapy, and chemotherapy, which can all lead to significant side effects. The goal of radiation therapy is to target the tumor with a high dose of radiation while minimizing the impact on surrounding healthy tissues. To address the potentially harmful effects of radiation therapy, one approach is to use methods that make cancer cells more sensitive to radiation. Chemotherapy drugs can help increase this sensitivity but also come with side effects like bone marrow suppression, increased mucus production, and skin inflammation(26). To enhance the damage caused by radiation to cancer cells while minimizing the impact on normal tissues, scientists have been studying substances that target cancer cells specifically and heighten their sensitivity to ionizing radiation(27). Radiosensitizers refer to medications or chemical compounds that amplify the lethal effects of radiation. A reliable radiosensitizer must exhibit positive therapeutic effects, meaning it should have a differing impact on tumors compared to normal tissues for clinical utility(28). The mechanism behind increased radiation sensitivity includes multiple factors such as hindering the repair of radiation-induced damage, altering the signaling pathways of tumor cells, initiating programmed cell death, or modifying cell metabolism(29). However, in recent years, natural-based remedies have emerged as a potential alternative treatment option (30). Quercetin, a powerful flavonoid with anti-inflammatory and anti-cancer properties, can be found in various fruits and vegetables such as citrus fruits, apples, radish leaves, and red onions. Previous studies have shown that quercetin exhibits cytotoxic effects on numerous types of cancer cells (31). Our study aimed to investigate the in-vitro radiosensitizing effect of quercetin on MCF-7 breast cancer cell line. To achieve this, we utilized the micronucleus test and colony assay to examine the toxicity induced by quercetin on the cancer cells. We also assessed the impact of this compound on the cells' antioxidant properties by analyzing the activity of superoxide dismutase and catalase enzymes.

As far as we are aware, this study is the first to explore different doses of quercetin pretreatment in irradiated MCF-7 cell lines. Our study revealed that pretreatment with different doses of quercetin enhances the sensitivity of irradiated MCF-7 cell lines to radiation. This results in significantly higher genotoxicity and reduced cell survival compared to the control groups that were only irradiated. In the quercetin-treated groups, the number of micronuclei increases while the number of colonies decreases. Nevertheless, the cells that received quercetin treatment and were exposed to radiation exhibited a significantly lower cell survival rate compared to the group that was only exposed to radiation.

So far, various studies have investigated the different concentrations of quercetin potential on various cancer cells. In this regard, some have demonstrated that Quercetin inhibited the growth, migration, and invasion and induced apoptosis of by antagonizing SHH and TGF-β/Smad signaling pathways. Thus, quercetin may be a potential candidate for Pancreatic ductal adenocarcinoma treatment (32, 33). In a similar study, researchers investigated the effect of quercetin on oxidative stress caused by ultraviolet A (UVA) radiation in rats. Exposure to UVA rays can lead to the production of reactive species and damage to cell components. The rats were divided into three groups: control, exposed to UVA, and exposed to UVA and treated with quercetin (50 mg/body weight). The results showed that the enzyme activities of glutathione peroxidase, glutathione reductase, catalase, and superoxide dismutase decreased significantly after irradiation. However, in the group treated with quercetin, all of these enzyme activities were significantly higher than in the group exposed to irradiation alone, indicating that quercetin has a protective effect (34). Another similar study investigated the protective effect of quercetin against oxidative stress caused by ultraviolet radiation. Again,

rats were divided into three groups: control, ultraviolet-exposed, and ultraviolet-exposed with quercetin (50 mg/g body weight). In the group exposed to ultraviolet radiation with quercetin, the enzyme activity of catalase and superoxide dismutase was significantly higher than in the group exposed to ultraviolet radiation alone, reinforcing quercetin's potential protective effect (35). The results of the present study showed that the activity of catalase and superoxide dismutase did not change significantly between the control group and groups that were given different amounts of quercetin (20, 40, and 60 μM). However, radiation increased the level of reactive species in cells and depleted the storage of antioxidant enzymes (catalase and superoxide dismutase). The study discovered that the activity of catalase and superoxide dismutase was significantly different in the group that was exposed to 2 and 3 Gy radiation compared to the control group. This suggests that the radiation caused more oxidative stress. In contrast to previous research, the groups that were given quercetin and then exposed to radiation (2 and 3 Gy) had significantly different levels of the enzymes catalase and superoxide dismutase compared to the group that was only exposed to radiation. The levels of these enzymes were lower in the groups that were given quercetin and then radiation. This suggests that quercetin may act differently in cancer cells compared to normal tissue, causing a reduction in these vital enzymes in cancer cells.

In a similar study, the result showed that 40 μM quercetin significantly reduced the number of MCF-7 cells (36). Additionally, Li et al. demonstrated in 2018 that quercetin at 50 IC50 μM experimentally reduced the survival rate of MCF-7 cells (37). Also, Niazvand et al.'s study found that solid lipid nanoparticles containing 25 μmol of quercetin lowered the number of MCF-7 cells by stopping their growth and killing them (38). In our study, we saw that quercetin at 40 and 60 μM greatly increased the number of micronuclei compared to the control group, which was made up of MCF-7 cells that had not been treated with quercetin. An increase in the number of micronuclei indicates damage to the DNA molecule, which ultimately leads to cell death. Also, counting the colonies showed that the number of colonies was much lower in the group that was given 40 and 60 μM quercetin compared to the control group. This results confirms that the survival rate of cells treated with quercetin has decreased.

In a different study, researchers investigated the effects of quercetin on the cellular response to ionizing radiation in the HepG2 cell line. They used gamma rays at 1, 5, and 10 Gy, along with quercetin at concentrations of 10, 20, 40, 80, and 100 μM. The findings showed that cell survival decreased after a 24-hour treatment with quercetin. Additionally, the cell survival rate was significantly lower in the group treated with both quercetin and ionizing radiation compared to the group treated with quercetin alone. The combined treatment of quercetin and ionizing radiation also reduced the activity of catalase and superoxide dismutase. These results suggested that combining quercetin with ionizing radiation could enhance the efficacy of radiation therapy (39). In our study, like the research mentioned above, quercetin increased the effect of ionizing radiation on the studied cells (MCF-7), which was associated with an increase in the number of micronuclei and a decrease in colonies. Ionizing radiation causes DNA damage through the generation of active species and direct effects, ultimately resulting in cell death. This genetic damage leads to an increase in the number of micronuclei, indicating cellular damage. On the contrary, radiation caused a greater reduction in the activity of two enzymes, catalase and superoxide dismutase, in the groups treated with both quercetin and radiation compared to the group treated with radiation alone. The decline in the activity of these two antioxidant enzymes was attributed to the oxidative stress induced by quercetin and ionizing radiation on cancer cells. In addition, quercetin has been shown to enhance the effects of radiation in MDA-MB-231 breast cancer cells (36), which further supports the idea that quercetin's radiosensitizing effects may be generalizable across different breast cancer subtypes. Similar to our findings, other studies have shown that quercetin reduces cell survival and increases DNA damage when combined with radiation, suggesting that quercetin may play a role in preventing DNA repair in cancer cells, thereby amplifying radiation-induced cell death.

Research conducted by Lin et al. in 2008 demonstrated that the presence of 40 micromoles of quercetin significantly decreased the number of MCF-7 cells

(40). Additionally, in 2018, Li et al. revealed that quercetin with an IC50 of 50 micromoles effectively reduced the viability of MCF-7 cells(41). In our investigation, we found that quercetin at concentrations of 40 and 60 μM led to a noticeable increase in the quantity of micronuclei, differing significantly from the control group (MFC-7 cells that weren't treated with quercetin). An upsurge in micronuclei is indicative of DNA damage, which could ultimately result in cell death. Furthermore, the assessment of colony quantity illustrated that in the 40 and 60 micromolar quercetin-treated groups, the number of colonies was markedly lower than in the control group, reaffirming the decrease in cell survival rate following quercetin treatment.

In 2022, Askar and colleagues conducted a study exploring the impact of combining quercetin nanoparticles with targeted radiation therapy for treating breast cancer. in vitro research demonstrated that incubating MCF-7, Hepg-2, and A459 cancer cells with quercetin nanoparticles for 24 hours resulted in the inhibition of cancer cell growth. Furthermore, the combination of quercetin nanoparticle treatment with radiation therapy effectively suppressed the proliferation of MCF-7 cancer cells. During the in vivo phase, female albino mice with breast cancer exhibited inhibited tumor growth and significantly enhanced response to radiotherapy when treated with quercetin nanoparticles. Consequently, the study concluded that the combination of quercetin nanoparticles and radiation therapy could serve as an effective treatment approach for controlling and treating breast cancer(10). Our study findings were in line with Askar et al.'s research. We observed a significant increase in the number of micronuclei in the quercetin group treated with radiation compared to the radiation-only group. An elevated number of micronuclei indicates damage to the cell's genetic material. Additionally, we conducted colony counting alongside the micronucleus assay to further elucidate quercetin's effect. A comparison of the number of colonies in the two aforementioned groups revealed that quercetin caused a more pronounced reduction in colony count. Consequently, the survival rate of the breast cancer cell line (MCF-7) in the group treated with quercetin and radiation was lower than that in the radiation-only group.

Comparing our results with those of other well-established radiosensitizers, such as cisplatin and gemcitabine, also reveals interesting insights. Both cisplatin and gemcitabine have been

extensively studied for their ability to enhance the effects of radiation, and their mechanisms of action include interference with DNA repair and cell cycle progression (42). Quercetin shares some of these mechanisms, including the inhibition of antioxidant enzymes and the induction of oxidative stress (34, 35), which may contribute to its radiosensitizing effects. However, unlike cisplatin and gemcitabine, which are cytotoxic to both cancer and normal cells, quercetin appears to be more selective, potentially causing less toxicity to normal tissues (28). This selective toxicity could make quercetin a promising candidate for further development as a radiosensitizer, especially when combined with targeted radiation therapy, as demonstrated by Askar et al. (10), who found that quercetin nanoparticles enhanced the radiosensitivity of MCF-7 cells both in vitro and in vivo.

In contrast to previous studies on quercetin’s effects in normal cells, our results suggest that quercetin may cause a reduction in antioxidant enzyme levels specifically in cancer cells, which may enhance the radiosensitizing effects of radiation in tumor cells. These findings suggest that quercetin’s impact on antioxidant defense systems is context-dependent, acting differently in cancer cells compared to normal tissues, and may contribute to its selective radiosensitizing properties.

Conclusion

In our current research, we examined the impact of quercetin on the MCF-7 breast cancer cell line under both non-radiated and radiated conditions. Overall, the findings of this study revealed that exposure to ionizing radiation leads to cellular damage and decreased survival rates in breast cancer cell lines, resulting in elevated micronuclei levels and reduced colony numbers compared to the control group. Treatment with quercetin produced similar outcomes. However, in cells treated with quercetin and exposed to radiation, the cell survival rate was notably lower than in the group subjected solely to radiation. Moreover, the levels of superoxide dismutase and catalase enzymes in

quercetin-treated cells exposed to radiation quercetin were significantly lower than those in the group subjected only to radiation.

While the findings of this study suggest that quercetin can act as a potent radiosensitizer in MCF-7 breast cancer cells, there are several limitations that need to be acknowledged. First, the current study was performed exclusively in vitro using the MCF-7 cell line, which represents only one subtype of breast cancer. The results may not be fully representative of the diverse molecular and genetic characteristics present in other breast cancer subtypes or in tumors from different patients. Therefore, additional studies utilizing a broader range of breast cancer cell lines, including triple-negative breast cancer (TNBC) or HER2-positive subtypes, would provide a more comprehensive understanding of quercetin's radiosensitizing potential across different cancer types.

Second, although we observed significant effects of quercetin on cell survival and genotoxicity, the underlying molecular mechanisms responsible for its radiosensitizing effects need further exploration. For example, while our study focused on the alteration of antioxidant enzyme activity, it would be valuable to investigate the effects of quercetin on other key signaling pathways involved in DNA damage repair, cell cycle regulation, and apoptosis.

The next logical step would be to investigate quercetin’s radiosensitizing effects in in vivo models, where tumor growth, metabolism, and drug bioavailability can be better assessed in the context of the entire organism. Animal studies, particularly in mouse xenograft models of breast cancer, would provide a more accurate reflection of how quercetin interacts with radiation in a living system, including its pharmacokinetics and potential toxicity.

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Ethical approval

All the experimental procedures in this study were approved by the Ethics Committee of Guilan University of Medical Sciences, Rasht, Iran (ethical code IR.GUMS.REC.1402.619).

Author contribution

MHZ Conceptualization, editing, review, and supervision. **MB** Written and Laboratory tests. **HSS** Radiation therapy.

Conflict of interest

There is no Conflicts of interest/competing interests.

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