Iranian Journal of Medical Physics

ijmp.mums.ac.ir

Investigating Direct and Bystander Radio-Sensitizing Effects of Genistein in PC-3 Prostate Cancer Cells

Reza Fardid [1*], Aysuda Mansuri [1], Mohammad Ali Takhshid [2], Malihe Rostamyari [1], Seyedeh Kimia Javadi Haghighi[1], Mohammad Amin Mosleh-Shirazi [3]

1. Department of Radiology, School of Paramedical Sciences, Shiraz University of Medical Sciences, Shiraz, Iran
2. Diagnostic Laboratory Sciences and Technology Research Center, School of Paramedical Sciences, Shiraz University of Medical Sciences, Shiraz, Iran
3. Department of Radio-oncology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

ARTICLE INFO

Article type:
Original Paper

Keywords:
Genistein
Radiation
Bystander Effect
Prostatic Neoplasms

ABSTRACT

Introduction: Radiation therapy is the major treatment modality of prostate cancer. Genistein (GEN) as the most abundant isoflavone in soybeans prevents the repair of DNA breaks, improving the radiosensitivity of cancer cells. It is demonstrated that cancer cells can be eradicated outside the radiation field as a result of the bystander effect. The aim of this study was to investigate the direct and indirect effect of ionizing radiation (IR), GEN and their combination on prostate cancer cell line (PC-3).

Material and Methods: Cells were irradiated to 4 Gy IR 24h after treatment with GEN. Then the medium of cells was transferred to non-irradiated cells to induce bystander effect. The direct and indirect effects of GEN, IR, and their combination (GEN+IR) on cell cycle and apoptosis were assessed by flow-cytometry.

Results: Cell cycle arrest has occurred in the G2/M phase in the GEN and GEN+IR groups, while no significant differences were observed in the bystander groups. Apoptosis levels were also significantly increased by GEN, IR and combined treatment. Although apoptosis was significantly higher in Bys+IR group compared to control, indirect exposure to both GEN and IR did not enhance apoptosis levels.

Conclusion: Overall, the results demonstrate that GEN enhances PC-3 cells radiosensitivity by promoting apoptosis and cell cycle arrest. However, bystander effect was not observed by indirect exposure of PC-3 cells to both GEN and IR. Future research are crucial to explore the radiation-induced bystander effect in the presence of GEN.

►*Please cite this article as:*
Fardid R, Mansuri A, Takhshid MA, Rostamyari M, Javadi Haghighi SK, Mosleh-Shirazi MA. Investigating Direct and Bystander Radio-sensitizing Effects of Genistein in PC-3 Prostate Cancer Cells. Iran J Med Phys 2026; 23 (2): 86-92. 10.22038/ijmp.2026.92400.2642.

Introduction

Prostate cancer (PCa) is a major global health issue, representing the second leading cause of cancer mortality in men after lung cancer. In 2020, approximately 1.4 million new cases were diagnosed and 375,000 deaths occurred worldwide [1, 2]. According to the stage of PCa, a variety of primary treatments are available, including surgery, radical prostatectomy, chemotherapy, radiotherapy, androgen-deprivation therapy, and cryotherapy [3], of which radiotherapy is more commonly used for primary treatment. Radiation causes DNA damage that exceeds the repair capacity of cells, leading to apoptosis, cell cycle arrest, and autophagy [4]. However, high radiation doses are limited by potential toxicity to surrounding tissues. Some techniques like three Dimension Conventional Radiation Therapy (3D-CRT) and Intensity Modulated Radiation Therapy (IMRT) have improved outcomes by enabling higher doses, better tumor targeting, and normal tissue protection [5]. In spite of these technologies, radioresistant cancer cells, local recurrences, and distant metastases following radiation therapy make it important to investigate new approaches for enhancing its effectiveness [6].

Chemicals or pharmaceutical substances known as radiosensitizers can increase the sensitivity to radiation of cancer cells and enhance the killing effect on tumor cells by altering the DNA sensitivity to radiation, disrupting DNA repair processes, causing free radicals indirectly, and arresting cells at critical phases of their life cycle [7]. Chemotherapy drugs are often used as adjuvants in order to optimize the clinical outcome of radiotherapy, However, these drugs are not favored due to several limitations, including, poor solubility, lack of specificity, and short circulation [8]. Nowadays, natural radiosensitizers including soy isoflavones (SIF) are preferred as they can inhibit the proliferation and tumorigenic activity of PCa cells while also enhancing their radiation sensitivity [9].

GEN (4', 5, 7-trihydroxyisoflavone) as the most abundant SIF in soybeans has a polyhydroxy

*Corresponding Author: Tel: +96 912 388 4826; Email: rfardid@gmail.com, rfardid@sums.ac.ir

polyphenol structure of ketones or non-ketones [10]. In recent years, many studies have been performed on a wide variety of pharmacological characteristics of GEN, including antioxidant, anti-inflammatory, anti-angiogenic, pro-apoptotic, and anti-proliferative capabilities in the prevention and treatment of several types of cancers [11-16]. Besides its Phyto-estrogenic properties, GEN structurally mimics the mammal's primary estrogen, 17β-Estradiol (E2) which enables interaction with estrogen receptors [17]. Distinctive characteristics of GEN, including having a strong binding affinity specifically to the β estrogen receptor, in contrast to its relatively weaker binding affinity to α estrogen receptors, could potentially impact estrogen metabolism and inhibit the growth of hormone-related cancers [18, 19]. Additionally, GEN inhibits tyrosine and histidine kinases as well as topoisomerase I and II, preventing DNA repair pathways and improving the radiosensitivity of cancer cells [20, 21].

Radiation-induced bystander effect (RIBE) refers to radiation biological effects in a population of cells that are not directly exposed to radiation but are located adjacent to or even farther away from the irradiated cells [22], leading them to exhibit similar effect to directly exposed cells such as heightened genomic damage, DNA damage, altered apoptosis, reduced clonogenic survival and oncogenic transformation [23]. RIBE occurs through gap junction communication, soluble factors in the culture medium, and complex signaling involving inflammatory cells, oxidative stress, free radicals, and immune pathways [24]. While studies have shown that RIBE is more pronounced in fractionated doses, this effect has also been documented for cytotoxic drugs [25, 26]. It may contribute in processes such as tumor recurrence, metastasis, and the development of secondary cancers, such as increased lung cancer risk following prostate radiotherapy through signalling pathways in distant tissue [27]. In contrast, other studies have highlighted the potential therapeutic efficacy of the RIBE by transferring culture medium from irradiated cells to non-irradiated cells resulting in reduced survival [28].

Previous studies have shown that both GEN and x-ray radiation induce apoptosis in PCa cells through direct exposure [29-32]. However, none of these studies have examined the bystander effect of this combined therapy on cell cycle progression and apoptosis of PC-3 cells so far. In this study, we examined the effects of direct and indirect exposure to GEN, x-ray radiation, and their combination on cell cycle changes and apoptosis in PC-3 cell line.

Materials and Methods

Cell Culture Materials

The human prostate cancer cell line was obtained from the National Cell Bank of Iran (Pasteur Institute, Iran) and cultured in RPMI 1640 (Shellmax) supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco) and 1% (v/v) penicillin-streptomycin (Shellmax). The cells were incubated in a humidified incubator at 37 °C with 5% CO_2. GEN (molecular weight: 270.241 g/mol, storage temperature: -20 °C) was purchased from Sigma Aldrich and dissolved in DMSO (Sigma). A stock solution at a concentration of 0.03 M GEN was prepared and stored at -20 °C. To achieve the required concentration, the main stock was diluted using the culture medium. The cells in the placebo group were treated with a dilution equivalent to DMSO in the culture medium.

GEN Cytotoxicity Analysis

Cytotoxicity effect of different concentrations of GEN was evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. PC-3 cells (15×10^3 cells/well) were seeded in 96-well cell culture plates to assess the cytotoxicity of GEN. After overnight incubation to allow cell attachment, cells were treated with GEN at various concentrations (20, 45, 70, 90, 120, and 150 µM), and placebo which contained the equivalent percentage of DMSO as the highest concentration of GEN which was less than 0.3% DMSO. 48h after treatment, the culture medium was replaced with 0.5% MTT solution dissolved in phosphate-buffered saline (PBS) and then incubated for four hours. After that, 100 µl DMSO was used to dissolve the formazan crystals. The optical intensity of each well was measured at 570 nm with a microplate ELISA reader (Stat Fax-2100, Awareness Technology, Inc. USA).

X-ray exposure set up

In this study, a total dose of 4 Gy was delivered to PC-3 cells cultured in T12.5 flasks using a 6 MV Electa compact linear accelerator (Crawly, UK) located at the department of Radiotherapy at Namazi Hospital, Shiraz, Iran, at a dose rate of approximately 200 MU/min. The cell culture flasks were placed at 100 cm distance from the source (SSD: source-surface distance) in a radiation field size of 25 cm × 25 cm with gantry angle of 0°. To ensure sufficient lateral, forward, and back scattering of radiation to achieve balanced charged-particle equilibrium conditions, 5 square Plexiglas plates with a size of 900 cm^2 and a thickness of 1 cm below and one above the cell culture flasks were used.

Experimental Protocol

In order to evaluate the direct and bystander effects of GEN and IR in PC-3 cells, 7 experimental groups were defined: 1) Control, 2) Placebo: treatment with the highest concentration of the drug solvent (>0.3% DMSO), 3) IR: 4 Gy exposure to PC-3 cells, 4) GEN: treatment with GEN, 5) GEN+IR: cells exposed to 4 Gy IR 24h after GEN treatment, 6) Bys+IR: cells received IR through the bystander effect and 7) Bys+GEN+IR: cells received 4 Gy IR following GEN treatment through the bystander effect.

24h after cells were exposed to IR, the culture medium of IR and GEN+IR groups were collected and replaced with fresh medium. To induce the bystander effect, the collected medium was centrifuged twice at 1500 rpm for 5 minutes to ensure the absence of any cell in the medium and then transferred to two flasks that had been seeded 48h ago, as Bys+IR and Bys+GEN+IR groups and incubated for 24h before each experiment. The timings have been scheduled to ensure that the duration from seeding to the initiation of each experiment is uniform for all groups, lasting for 72 hours. This standardized duration guarantees that all cellular groups have approximately similar Population Doubling Time (PDT of PC-3 cells= 33hours [33]) at the onset of each experiment.

Cell Cycle Analysis

PC-3 cells at a density of 60×10^4 cells were cultured in T12.5 flasks. All treated groups and their corresponding controls were harvested 72 h after seeding, fixed with cold 70% ethanol and stored overnight at 4°C. After washing off ethanol, cell pellets were suspended in a combination of PBS and 0.25% Triton X-100 and incubated on ice for 15 minutes. Cells were centrifuged at 4000 rpm for 2 minutes before incubation with RNase A (10 µg/ml, CinnaGen) and PI (4 µg/ml). Finally, after room temperature incubation in the dark for 30 minutes, flowcytometry (BD FACSCalibur) was employed to assess the distribution of cell cycle phases in PI-stained cells, and the data were analyzed using FlowJo software, V10 (FlowJo, LLC).

Apoptosis Analysis

Apoptosis levels were measured by Flowcytometry (BD FACSCalibur) with Annexin V-FITC / PI Apoptosis Detection Kit (Baran Biotech, Cat No. B24081-25). Briefly, PC-3 cells had been cultivated with a density of 6×10^4 cells in a T12.5 cell culture flask. After irradiation and drug treatment, cells were trypsinized and then washed twice with PBS and suspended in 500 µl of annexin V binding solution (1X). The resulting solution was centrifuged, and the supernatant was discarded, and resuspended in 100 µl of binding buffer 1X solution, and transferred to flow cytometer tubes. After that, 5 µl of Annexin V-FITC staining solution and 2 µl of PI staining solution were both added to the cell suspension, followed by incubation for 15 minutes at room temperature in the dark. For fluorescence compensation, four samples including unstained cells from untreated cells, Annexin V-stained cells, PI-stained cells and double-stained cells from positive control group which was treated with H_2O_2 for 30 minutes, were used to correct any spectral overlap between Annexin V-FITC and PI. After that, apoptosis was evaluated by flowcytometry and the resulting data was analyzed with FlowJo software 10. Gating strategy was based on defining over %90 of the live cells' population in negative control group.

Statistical analysis

In the present study, all data were reported as mean ± standard deviation of at least three parallel measurements of the experiment. To evaluate statistical significance among all groups, One-way ANOVA and Kruskal-Wallis tests were performed using GraphPad Prism 9, followed by Tukey or Dunn's post-hoc tests, respectively. P-value < 0.05 was considered statistically significant.

Results

The cytotoxicity of GEN in PC-3 cells

Figure 1 shows the cytotoxic effect of GEN at various concentrations (20, 45, 70, 90, 120, and 150 µM) on PC-3 cells obtained by the MTT cell toxicity assay. As it can be seen from the figure 1, <0.3% DMSO as placebo, did not show any toxicity on PC-3 cells and more than 99% of the cells remained viable after 48h of treatment. The viability of PC-3 cells decreased in a dose-dependent manner as the GEN concentration increased. 90 and 120 µM GEN nearly reduced the viability of PC-3 cells to 50%. Therefore, a concentration of 90 µM of GEN was used as a radiation sensitizer in all of the following conducted experiments.

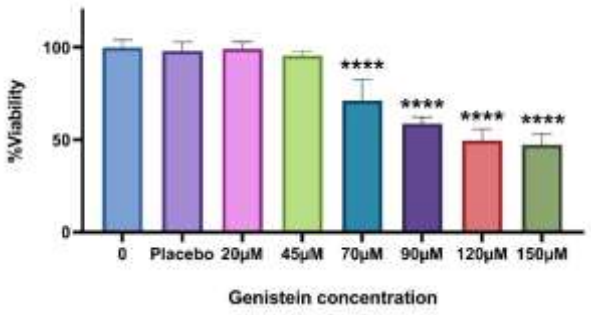

Figure 1. The %viability of PC-3 prostate cancer cells treated with Genistein at different concentrations for 48h. Placebo group contained the equivalent percentage of Dimethyl sulfoxide (DMSO) as the highest concentration of Genistein. (****: $p < 0.0001$)

Cell cycle alterations in PC-3 cells

Cell cycle progression of PC-3 cells was analysed in order to investigate the molecular mechanism involved in apoptosis enhancement by GEN, irradiation or both treatments. The distribution of cells in different phases of the cell cycle followed by different treatments are displayed in Figure 2. Percentage of cells in G0/G1 phase significantly decreased after GEN treatment compared to control and IR ($p<0.0001$). The highest density of cells in S phase belonged to GEN treated cells and GEN + IR ($p<0.0001$, $p=0.0001$ respectively). GEN, IR and GEN+IR significantly increased the cell population in G2/M phase, the sensitive phase of the cell cycle compared to control ($p<0.0001$).

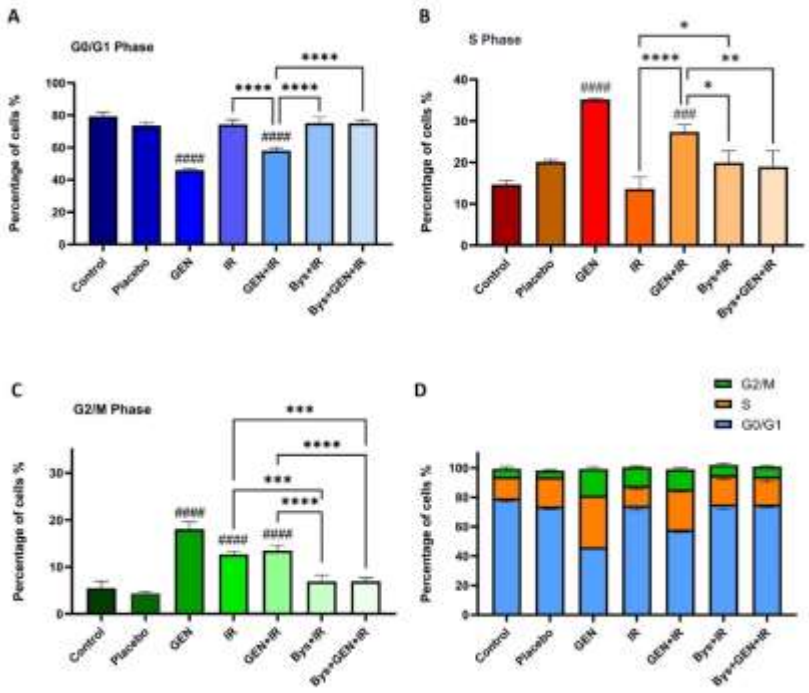

Figure 2. The distribution of PC-3 prostate cancer cells in different phases of the cell cycle followed by different treatment groups, A) G0/G1 phase, B) S phase, C) G2/M phase and, D) Distribution of PC-3 prostate cancer cells in phases of the cell cycle in different groups (data reported from three independent replicates as mean ± standard deviation, ###: p<0.001 and ####: p<0.0001 compared to control, *: p<0.05, **: p<0.01, ***: p<0.001, and ****: p<0.0001)

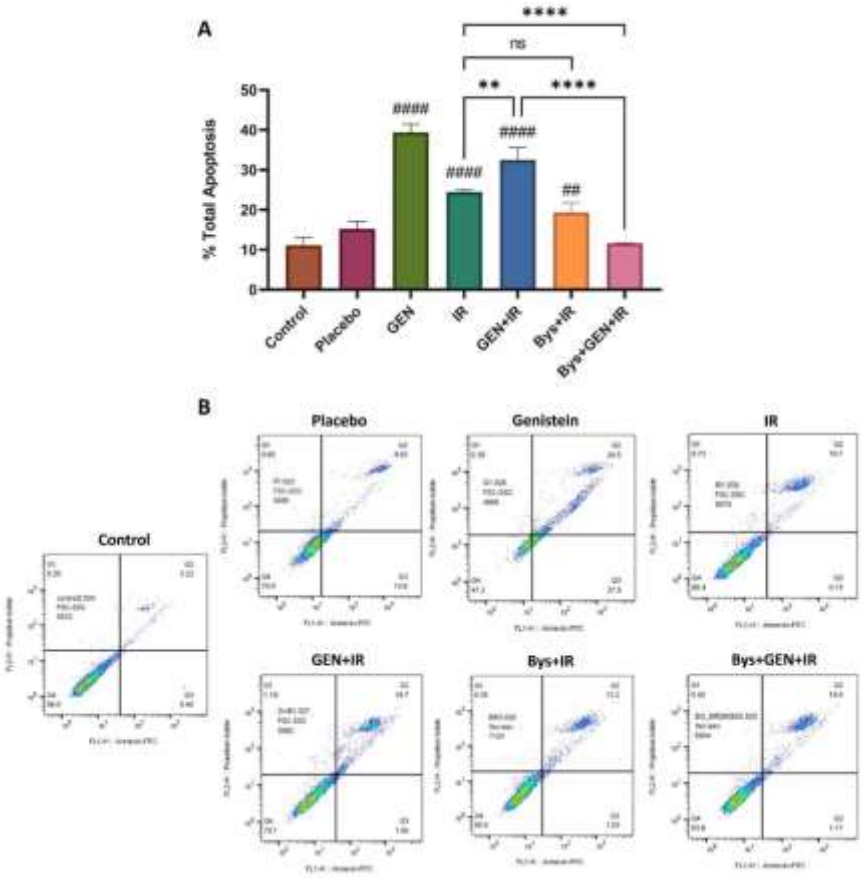

Figure 3. %Total apoptosis of PC-3 prostate cancer cell after treatment with Genistein and Ionizing Radiation (A), Flowcytometry patterns obtained from FlowJo 10 in different groups (B) (data reported from three independent replicates as mean ± standard deviation in different groups, ##: p<0.01 and ####: p<0.0001 compared to control, ns: non-significant (p>0.05), **: p<0.01 and ****: p<0.0001)

Apoptosis levels in PC-3 cells

Flowcytometry data showed increased apoptosis in all PC-3 cells across different groups of treatments compared to control with the highest values in GEN and GEN+IR groups ($p<0.001$, Figure 3). Compared to control, apoptosis levels were also significantly elevated in IR and Bys+IR groups ($p <0.001$ and $p=0.0024$, respectively). GEN enhanced radiation-induced apoptosis in cells that were exposed to both GEN and IR ($p=0.003$). Moreover, PC-3 cells which received GEN and IR indirectly through media transfer, showed significantly lower apoptosis than those who were directly exposed to GEN and IR ($p<0.001$), yet indirect radiation exposure did not significantly affect the apoptosis of the PC-3 cells ($p=0.0762$).

Discussion

Foods rich in SIF such as soybeans offer health benefits as they are both anti-inflammatory and antioxidant with DNA repair capacity [34]. Over the past years, numerous studies have been conducted on the preventive role of GEN, as the most common SIF, in various types of cancer particularly prostate cancer [35]. GEN possesses pro-apoptotic, anti-angiogenic and anti-metastatic [36] properties, making it suitable for utilization as a radiosensitizer in adjuvant therapy, leading to the inhibition of cancer cell growth.

In this study, PC-3 cells viability decreased (relative to untreated cells) in a dose-dependent manner, with a half maximal inhibitory concentration (IC_{50}) of approximately 90 µM (Figure 1). Hermann et al. demonstrated that pre-treating PC-3 cells with GEN (10 µM) followed by a 0.3 Gy radiation resulted in a 57% reduction in colony formation compared to unirradiated controls [37]. Other studies reported lower IC_{50} values, including 43 µM (Kyle et al [13]) and 50 µM (Shafiee et al [38]), while the viability of PC-3 cells also decreased to nearly less than %50 with 90 µM. While plasma concentration of GEN is about 1-5 µM from digesting soy foods [39], in vitro studies use higher concentrations of about 10-100 µM which are still considered biologically and physiologically safe with no evidence of harm from consumption of high doses of SIF compounds.

As shown in Figure 2A, all treated groups showed a slight reduction in G0/G1 cell density compared to control, with the GEN-treated group showing the lowest density (~33%, $p<0.0001$). This suggests that GEN may promote G1 progression in PC3 cells by activating cyclins, cyclin-dependent kinases (CDKs), or growth factor signaling. In the S phase, all treatments except radiation alone increased cell population in G0/G1, with the highest levels observed in the GEN group. Radiation did not reduce this GEN-induced increase, indicating that GEN may interfere with DNA replication process and activate S-phase checkpoints (Figure 2B). In the G2/M phase, cells treated with GEN, IR, or GEN+IR showed significant accumulation compared to control and placebo group ($p<0.001$, Figure 2C), suggesting G2/M arrest may be due to cell cycle checkpoint activation and enhanced DNA damage responses. Nevertheless, indirectly exposed PC-3 cells showed no significant changes across cell cycle phases, indicating the absence of bystander effect (Figure 2D).

These findings align with those reported by Raffoul et al. [21], showing G2/M cell cycle arrest following 3 Gy irradiation or GEN treatment. Hillman et al [30] also demonstrated that DNA synthesis and cell division can be inhibited by GEN (15 mM) when combined with radiation, possibly through inhibition of NF-κB signaling pathway. Additionally, Hermann et.al [37] reported that GEN or Estradiol did not significantly alter cell cycle distribution; however, 4 Gy irradiation resulted in an increased G2/M accumulation. Since G2/M is known to be a radiosensitive phase, these observations are relevant for radiosensitization of cancer cells by GEN [37].

In apoptosis assay, it was observed that apoptosis level was significantly elevated in cells directly exposed to GEN, IR, combination of GEN and IR and in cells indirectly exposed to IR compared to control which are as a result of G2/M cell cycle arrest in these groups. In agreement with our results, Davis et al [40] and Tang et al [41] reported a significant increase in apoptosis of PC-3 cells treated with 50 and 30 µM GEN. Famuyiwa et al [42] also reported that 70 µM GEN caused %43 apoptosis in LNCap prostate cancer cells. In contrast, ~22 µM GEN did not significantly cause apoptosis in PC-3 cells in Vodnik et al [43] study. According to recent research, apoptosis occurs at relevantly higher doses of GEN, usually between 50-200 µM [39].

Radiation-induced bystander effect occurs in nearby un-irradiated cells by stress signals produced by radiation-induced damaged cells. These stress signals involve oxidative stress from formation of ROS and free radicals, inflammatory cytokines such as IL-1β, IL-6, TNF-α and TGF-β and NF-κB which transfer by cellular gap junctions or extracellular vesicles [44, 45]. Recent studies showed that PC-3 cells exposed indirectly to radiation exhibited DNA damage and apoptosis [46, 47] which is in line with the results of this study. GEN have shown to boost DNA damage and induces cell cycle arrest and apoptosis in irradiated PC-3 cells, while on the other hand, attenuates RIBE by modulating the reactive oxygen species (ROS) production and inflammatory NF-κB/TGF-β signaling pathways in prostate cancer cells, which are involved in bystander effect induction [46, 48]. These effects mean that GEN could enhance radiation-induced DNA damage and apoptosis by inhibiting DNA repair systems and cellular pathways (like DNA-PKcs/Rad51) in directly irradiated cells, while alleviating the destructive factors involving in bystander effect in indirectly irradiated bystander cells [49]. The results of this study somehow revealed these effects as it was shown that apoptosis was significantly increased in indirectly irradiated PC-3 cells compared to control. However, PC-3 cells that were indirectly exposed to both GEN and IR, apoptosis were lower than those exposed directly to GEN and/or IR or indirectly to radiation alone, which can be as a result of GEN paradoxically actions. The GEN concentration

used in our study, as mentioned before, is much higher than GEN's physiological concentration in plasma. Therefore, it could limit the possibility to directly relate our in-vitro findings or even other recent experimental research that also used higher concentrations of GEN, to clinical applications of GEN in radiation therapy. For better clinical translation of any potential existence of a bystander effect, clinically relevant doses of GEN are recommended to be used in future in-vitro experiments. Nevertheless, according to the results of our study, inducing bystander effect in un-irradiated PC-3 cells did not significantly alter the cell cycle progression and apoptosis levels.

Conclusion

The findings of this study showed that GEN induced G2/M cell cycle arrest and apoptosis in PC-3 cells, both alone and in combination with radiation, suggesting that GEN could be a potential natural radiosensitizer to enhance radiotherapy efficacy against prostate cancer. Based on our study, indirect exposure of GEN and IR did not significantly impact the cell viability and progression of cell cycle, yet further studies are essential to explore the clinical utility of combined GEN and radiation treatment, and any potential bystander or abscopal effect, which may allow effective tumor killing while minimizing side effects and the possibility of metastasis.

Acknowledgment

This study was extracted from parts of a thesis written by A.M and was financially supported by Shiraz University of Medical Sciences under grant No. 24021. The authors express their gratitude to the Diagnostic Laboratory Sciences and Technology Research Center for their valuable contributions.

References

1. Culp MB, Soerjomataram I, Efstathiou JA, Bray F, Jemal A. Recent global patterns in prostate cancer incidence and mortality rates. Eur Urol. 2020;77(1):38-52.
2. Wang L, Lu B, He M, Wang Y, Wang Z, Du L. Prostate cancer incidence and mortality: global status and temporal trends in 89 countries from 2000 to 2019. Front Public Health. 2022;10:811044.
3. Gomella LG, Johannes J, Trabulsi EJ. Current prostate cancer treatments: effect on quality of life. Urology. 2009;73(5):S28-S35.
4. Borrego-Soto G, Ortiz-López R, Rojas-Martínez A. Ionizing radiation-induced DNA injury and damage detection in patients with breast cancer. Genet Mol Biol. 2015;38:420-32.
5. Li G, Li Y, Wang J, Gao X, Zhong Q, He L, et al. Guidelines for radiotherapy of prostate cancer (2020 edition). Precis Radiat Oncol. 2021;5(3):160-82.
6. Kaliberov SA, Buchsbaum DJ. Cancer treatment with gene therapy and radiation therapy. Adv Cancer Res. 2012;115:221-63.
7. Kayani Z, Islami N, Behzadpour N, Zahraie N, Imanlou S, Tamaddon P, et al. Combating cancer by utilizing noble metallic nanostructures in combination with laser photothermal and X-ray radiotherapy. J Drug Deliv Sci Technol. 2021;65:102689.
8. Zahraie N, Perota G, Vais RD, Sattarahmady N. Simultaneous chemotherapy/sonodynamic therapy of the melanoma cancer cells using a gold-paclitaxel nanostructure. Photodiagnosis Photodyn Ther. 2022;39:102991.
9. Palacios DA, Miyake M, Rosser CJ. Radiosensitization in prostate cancer: mechanisms and targets. BMC Urol. 2013;13(1):1-13.
10. Dixon RA, Ferreira D. Genistein. Phytochemistry. 2002;60(3):205-11.
11. Spagnuolo C, Russo GL, Orhan IE, Habtemariam S, Daglia M, Sureda A, et al. Genistein and cancer: current status, challenges, and future directions. Adv Nutr. 2015;6(4):408-19.
12. Kim JW, Kim JM, Kim S-K, Kim Y-M, Choi J-S. Protective effect of ginseng on salivary dysfunction following radioiodine therapy in a mouse model. Thyroid. 2018;28(8):1034-41.
13. Kyle E, Neckers L, Takimoto C, Curt G, Bergan R. Genistein-induced apoptosis of prostate cancer cells is preceded by a specific decrease in focal adhesion kinase activity. Mol Pharmacol. 1997;51(2):193-200.
14. Pagliacci M, Smacchia M, Migliorati G, Grignani F, Riccardi C, Nicoletti I. Growth-inhibitory effects of the natural phyto-oestrogen genistein in MCF-7 human breast cancer cells. Eur J Cancer. 1994;30(11):1675-82.
15. Spinozzi F, Pagliacci MC, Migliorati G, Moraca R, Grignani F, Riccardi C, et al. The natural tyrosine kinase inhibitor genistein produces cell cycle arrest and apoptosis in Jurkat T-leukemia cells. Leuk Res. 1994;18(6):431-9.
16. Alhasan SA, Pietrasczkiwicz H, Alonso MD, Ensley J, Sarkar FH. Genistein-induced cell cycle arrest and apoptosis in a head and neck squamous cell carcinoma cell line. Nutr Cancer. 1999;34(1):12-9.
17. Sirtori CR, Arnoldi A, Johnson SK. Phytoestrogens: end of a tale? Ann Med. 2005;37(6):423-38.
18. Banerjee S, Li Y, Wang Z, Sarkar FH. Multi-targeted therapy of cancer by genistein. Cancer Lett. 2008;269(2):226-42.
19. Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, Van Der Saag PT, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β. Endocrinology. 1998;139(10):4252-63.
20. Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S-i, Itoh N, et al. Genistein, a specific inhibitor of tyrosine-specific protein kinases. J Biol Chem. 1987;262(12):5592-5.
21. Raffoul JJ, Wang Y, Kucuk O, Forman JD, Sarkar FH, Hillman GG. Genistein inhibits radiation-induced activation of NF-κB in prostate cancer cells promoting apoptosis and G2/M cell cycle arrest. BMC Cancer. 2006;6(1):107.
22. Burdak-Rothkamm S, Rothkamm K. Radiation-induced bystander and systemic effects serve as a unifying model system for genotoxic stress responses. Mutat Res Rev Mutat Res. 2018;778:13-22.
23. Heeran AB, Berrigan HP, O'Sullivan J. The radiation-induced bystander effect (RIBE) and its

connections with the hallmarks of cancer. Radiat Res. 2019;192(6):668-79.
24. Najafi M, Fardid R, Hadadi G, Fardid M. The mechanisms of radiation-induced bystander effect. J Biomed Phys Eng. 2014;4(4):163.
25. Mothersill C, Seymour C. Bystander and delayed effects after fractionated radiation exposure. Radiat Res. 2002;158(5):626-33.
26. Ewald J, Desotelle J, Almassi N, Jarrard D. Drug-induced senescence bystander proliferation in prostate cancer cells in vitro and in vivo. Br J Cancer. 2008;98(7):1244-9.
27. Brenner DJ, Curtis RE, Hall EJ, Ron E. Second malignancies in prostate carcinoma patients after radiotherapy compared with surgery. Cancer. 2000;88(2):398-406.
28. Nh MZ, Abdullah R, Rahman W. Bystander effect induced in breast cancer (MCF-7) and human osteoblast cell lines (hFOB 1.19) with HDR-brachytherapy. J Biomed Phys Eng. 2020;10(3):319.
29. 29. Singh-Gupta V, Zhang H, Banerjee S, Kong D, Raffoul JJ, Sarkar FH, et al. Radiation-induced HIF-1α cell survival pathway is inhibited by soy isoflavones in prostate cancer cells. Int J Cancer. 2009;124(7):1675-84.
30. 30. Hillman GG, Forman JD, Kucuk O, Yudelev M, Maughan RL, Rubio J, et al. Genistein potentiates the radiation effect on prostate carcinoma cells. Clin Cancer Res. 2001;7(2):382-90.
31. 31. Raffoul JJ, Wang Y, Kucuk O, Forman JD, Sarkar FH, Hillman GG. Genistein inhibits radiation-induced activation of NF-κB in prostate cancer cells promoting apoptosis and G2/M cell cycle arrest. BMC cancer. 2006;6(1):1-10.
32. 32. Singh-Gupta V, Zhang H, Yunker CK, Ahmad Z, Zwier D, Sarkar FH, et al. Daidzein effect on hormone refractory prostate cancer in vitro and in vivo compared to genistein and soy extract: potentiation of radiotherapy. Pharm Res. 2010;27:1115-27.
33. 33. Cunningham D, You Z. In vitro and in vivo model systems used in prostate cancer research. J Biol Methods. 2015;2(1):e17.
34. 34. Norman HA, Butrum RR, Go VLW. Review of the international research conference on food, nutrition, and cancer, 2004. J Nutr. 2004;134(12):3391S-3S.
35. 35. Kim S-H, Kim C-W, Jeon S-Y, Go R-E, Hwang K-A, Choi K-C. Chemopreventive and chemotherapeutic effects of genistein, a soy isoflavone, upon cancer development and progression in preclinical animal models. Lab Anim Res. 2014;30(4):143-50.
36. 36. Lakshman M, Xu L, Ananthanarayanan V, Cooper J, Takimoto CH, Helenowski I, et al. Dietary genistein inhibits metastasis of human prostate cancer in mice. Cancer Res. 2008;68(6):2024-32.
37. 37. Hermann RM, Wolff HA, Jarry H, Thelen P, Gruendker C, Rave-Fraenk M, et al. In vitro studies on the modification of low-dose hyper-radiosensitivity in prostate cancer cells by incubation with genistein and estradiol. Radiat Oncol. 2008;3(1):19.
38. Shafiee G, Saidijam M, Tayebinia H, Khodadadi I. Beneficial effects of genistein in suppression of proliferation, inhibition of metastasis, and induction of apoptosis in PC3 prostate cancer cells. Arch Physiol Biochem. 2022;128(3):694-702.
39. Klein CB, King AA. Genistein genotoxicity: critical considerations of in vitro exposure dose. Toxicol Appl Pharmacol. 2007;224(1):1-11.
40. Davis JN, Singh B, Bhuiyan M, Sarkar FH. Genistein-induced upregulation of p21WAF1, downregulation of cyclin B, and induction of apoptosis in prostate cancer cells. Nutr Cancer. 1998;32(3):123-31.
41. Tang Q, Ma J, Sun J, Yang L, Yang F, Zhang W, et al. Genistein and AG1024 synergistically increase the radiosensitivity of prostate cancer cells. Oncol Rep. 2018;40(2):579-88.
42. Famuyiwa T, Boe A, Diaka J, Jebelli J, Esiobu N. Enhancement of genistein-induced apoptosis in LNCaP prostate cancer cells. J Cancer Prev Curr Res. 2016;4(2).
43. Vodnik VV, Mojić M, Stamenović U, Otoničar M, Ajdžanović V, Maksimović-Ivanić D, et al. Development of genistein-loaded gold nanoparticles and their antitumor potential against prostate cancer cell lines. Mater Sci Eng C. 2021;124:112078.
44. Buonanno M, Gonon G, Pandey BN, Azzam EI. The intercellular communications mediating radiation-induced bystander effects and their relevance to environmental, occupational, and therapeutic exposures. Int J Radiat Biol. 2023;99(6):964-82.
45. Smolarz M, Skoczylas Ł, Gawin M, Krzyżowska M, Pietrowska M, Widłak P. Radiation-induced bystander effect mediated by exosomes involves the replication stress in recipient cells. Int J Mol Sci. 2022;23(8):4169.
46. Pszczółkowska–Kępa B, Olejarz W, Głuszko A, Wałpuski G, Lorenc T, Brzozowska B. Exosome-mediated modulation of radioresistance: The radiation-induced bystander effect in prostate cancer cells. PLoS One. 2025;20(8):e0330501.
47. Tubin S, Valeriani M, Salerno G, Bracci S, Stoppacciaro A, Cardelli P, et al. Manipulation of radiation-induced bystander effect in prostate adenocarcinoma by dose and tumor differentiation grade: In vitro study. Int J Radiat Biol. 2015;91(2):166-71.
48. Liu X, Zheng T, Bao Y, Li P, Zhao T, Liu Y, et al. Genistein Implications in Radiotherapy: Kill Two Birds with One Stone. Molecules. 2025;30(1):188.
49. Ivashkevich A. The role of isoflavones in augmenting the effects of radiotherapy. Front Oncol. 2023;12:800562.