Gold nanoparticles and electroporation impose both separate and synergistic radiosensitizing effects in HT-29 tumor cells: an in vitro study

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Background and objective: Radiation therapy (RT) is the gold standard treatment for more than half of known tumors. Despite recent improvements in RT efficiency, the side effects of ionizing radiation (IR) in normal tissues are a dose-limiting factor that restricts higher doses in tumor treatment. One approach to enhance the efficiency of RT is the application of radiosensitizers to selectively increase the dose at the tumor site. Gold nanoparticles (GNPs) and electroporation (EP) have shown good potential as radiosensitizers for RT. This study aims to investigate the sensitizing effects of EP, GNPs, and combined GNPs-EP on the dose enhancement factor (DEF) for 6 MV photon energy.

Methods: Radiosensitizing effects of EP, GNPs, and combinations of GNPs-EP were comparatively investigated in vitro for intestinal colon cancer (HT-29) and Chinese hamster ovary (CHO) cell lines by MTT assay and colony formation assay at 6 MV photon energy in six groups: IR (control group), GNPs+IR, GNPs (24 h)+IR, EP+IR, GNPs+EP+IR, and GNPs (24 h)+EP+IR.

Results: Treatment of both cell lines with EP, GNPs, and combined GNPs-EP significantly enhanced the response of cells to irradiation. However, the HT-29 showed higher DEF values for all groups. In addition, the DEF value for HT-29 cells for GNPs+IR, GNPs (24 h)+IR, EP+IR, GNPs+EP+IR, and GNPs (24 h)+EP+IR was, respectively, 1.17, 1.47, 1.36, 2.61, and 2.89, indicating synergistic radiosensitizing effect for the GNPs (24 h)+EP+IR group. Furthermore, the synergistic effect was observed just for HT-29 tumor cell lines.

Conclusion: Combined GNPs-EP protocols induced synergistic radiosensitizing effect in HT-29 cells, and the effect is also tumor specific. This combined therapy can be beneficially used for the treatment of intrinsically less radiosensitive tumors.

Keywords: gold nanoparticles, radiosensitizer, electroporation, dose enhancement factor, synergistic effect

Introduction
Radiation therapy is the gold standard treatment option for more than half of cancer patients due to its ability to kill malignant cells and shrink tumors.1 It prevents tumor cell growth through bombardment of the tumor with ionizing radiation (IR). IR induces DNA damage by direct or indirect action through the generation of reactive oxygen species (ROS).2 Unfortunately, there is no discrimination between normal and malignant tissues in absorption of IRs, and thus doses of radiation must be limited to spare healthy surrounding tissue.3 One approach to increase discrimination between tumors and healthy tissues and thereby increasing the efficiency of radiation therapy is the use of radiosensitizer to preferably enhance dose at the site of tumor.4–6 In recent years,
various radiosensitizers have been developed to increase the outcome of radiation therapy. Material with high atomic number (Z) such as gold nanoparticles (GNPs)\textsuperscript{7,8} and physical approaches such as electroporation (EP)\textsuperscript{9,10} are two important examples to achieve this goal.

GNPs have been previously shown to improve the effect of radiotherapy in vitro\textsuperscript{11,12} and in vivo.\textsuperscript{13,14} GNPs have several characteristics that make them attractive for using with radiation therapy including small size (1–100 nm), biocompatibility, preferential passive accumulation in tumor, and the feasibility of surface modification to actively target cancerous cells.\textsuperscript{15–18} GNPs with high Z increase the cross section of photoelectric absorption and pair production interaction in the keV and MeV energy range, respectively, and thus enhance the delivering dose to the target tumor.\textsuperscript{19,20} Moreover, the interaction of X-ray with GNPs can release free radicals, thereby damaging DNA. Furthermore, GNPs have the ability to enter the mitochondria and induce apoptotic death.\textsuperscript{21}

EP is a physical process through which applying short intense electric pulses increases cell membrane permeability.\textsuperscript{22–24} In normal physiological conditions, the electric conductivity of cytoplasm and extracellular medium is much higher than the conductivity of the cell membrane. Thus, when an external electric field is applied to this lipid membrane, the anode-facing and cathode-facing side become hyperpolarized and depolarized, respectively, and a transmembrane potential is induced on the exposed cell.\textsuperscript{25,26} Equation (1) is generally used to describe this induced transmembrane potential:

$$\Delta V_m = f E_{ext} r \cos \phi$$

(1)

where $V_m$ denotes transmembrane potential, $f$ a factor that describes the effect of the cell on the extracellular field distribution, $E_{ext}$ the external electric field, $r$ the radius of cell and $\phi$ is the polar angle with respect to the external field. If $\Delta V_m$ is larger than a critical value (0.2–1.0 V), the EP is occurred, and the nanoscale pores are appeared in membrane.\textsuperscript{27,28} This phenomenon depends on pulse parameters such as amplitude, pulse frequency, pulse duration and number of pulse, and also on experimental conditions, for example, osmotic pressure, temperature, and conductivity of EP buffer, etc.\textsuperscript{27,29} If these electric parameters are chosen properly, the process of EP is reversible,\textsuperscript{30} and upon further increase of the electric parameters, the EP phenomenon becomes irreversible, which kills the cells.\textsuperscript{31,32} EP is routinely employed to transport nonpermeant molecules such as DNA, dyes, proteins, and chemotherapeutic drugs into the cell.\textsuperscript{33–35} However, it has been demonstrated that EP can induce oxidative jump and generate ROS.\textsuperscript{36} Gabriel and Teissie\textsuperscript{37} have reported that the generation of ROS is not homogenous and restricted to the electropermeabilized side of the cell membrane. Therefore, this technique can be combined with IR as a radiosensitizer to enhance the outcome of radiation therapy. West\textsuperscript{38} demonstrated that use of EP prior to irradiation can enhance the effect of irradiation by factor of 1.19.

Previous studies have demonstrated that EP and GNPs have radiosensitizing effects. However, we could not find any study investigating concurrent application of EP and GNPs to increase the sensitivity of cells to IR. Therefore, the present study was designed aiming to investigate the effects of EP and GNPs alone and in combination to increase the efficiency of radiation therapy. We hypothesized that the combination of EP and GNPs would induce synergistic radiosensitizing effect because of the following reasons:

1) EP can increase the uptake of GNPs by cells.
2) The conductivity of EP buffer is increased using GNPs, and this decreases the electric voltage consumed by the EP buffer, thereby improving the efficiency of EP.\textsuperscript{36}
3) GNPs act as microelectrodes\textsuperscript{35} and the electropermeabilization of membrane and thereby generation of ROS occurs on different sites of membrane.

**Materials and methods**

**Cell culture**

Colorectal cancer (HT-29) and Chinese hamster ovary (CHO) cell lines were purchased from National Cell Bank of Pasteur Institute of Iran (NCBI, C466 and C111) and grown as monolayers in Roswell Park Memorial Institute (RPMI) 1640 medium (BIO-IDEA, B11031, Tehran, Iran) enriched with 10% fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific, Waltham, MA, USA) and 1% penicillin/streptomycin (BIO-IDEA). The cells were routinely subcultured twice a week and maintained at 37°C in a humidified atmosphere with 5% CO\textsubscript{2} in an incubator (RS Biotech Galaxy R, West Lothian, UK).

**GNPs characterization**

GNPs (99.95+, 15 nm) were purchased from US Research Nanomaterials, Inc. (Houston, TX, USA). To prepare stock solution, the nanoparticles were suspended in deionized water, and other dilutions were performed in culture media immediately before use. The size and morphology of GNPs were estimated by transmission electron microscope (TEM) and scanning electron microscope. According to these
images, GNPs were spherical, and the average size was 12–15 nm in diameter (Figure 1A and B). We used GNPs at concentration of 0.1 mM.

**Study protocol**

To evaluate the radiosensitizing effect of GNPs, EP, and combinations of GNPs and EP, six different experimental groups were designed in this study (Figure 2): irradiation alone as a control group (IR), cells treated with GNPs immediately before irradiation (GNPs+IR), cells incubated with GNPs 24 h prior to irradiation (GNPs [24 h]+IR), cells exposed electric pulse 10 min before irradiation (EP+IR), cells treated with both GNPs and EP 10 min before irradiation (GNPs+EP+IR), and cells incubated with GNPs for 24 h and then receiving EP 10 min prior to irradiation (GNPs [24 h]+EP+IR). Different combinations of EP-GNPs with respect to time intervals between each modality were designed to study some important hypotheses on the mechanisms of actions of each modality.

**Electroporation set up**

The sample solution was dispensed into a 1 MM gap cuvette, and a single square pulse with voltage to distance ratio of 1,200 V/cm and 100 µs duration was delivered using a Bio-Rad Gene Pulser Xcell™ (Hercules, CA, USA) EP system at room temperature (21°C). Immediately after EP, the suspension was transferred either to 6-well or 96-well plate based on the assay, and then fresh culture medium was added to the cells. Finally, the plate was irradiated with ionization radiation after 10 min.

**Irradiation setup**

The cells were irradiated with megavoltage X-ray (6 MV) using Varian 2100 C/D linear accelerator (Golestan Hospital, Ahvaz, Iran) at a dose rate of 3 Gy/min with a field size of 20×20 cm². We used a Plexiglass (water equivalent) sheet with 1.5 cm thickness on top the plate (6-well in clonogenic cell survival assay and 96-well plate in MTT assay) as a build up to produce transient charged particle equilibrium. Moreover, another Plexiglass sheet with the thickness of 6 cm was placed under the bottom of plate to sufficient production of backscatter.

**Evaluation of radiosensitizing effect of GNPs, EP, and GNPs-EP**

**MTT assay**

The cellular response to each treatment at radiation doses of 2 and 4 Gy was assessed using MTT assay kit according to the manufacturer’s protocol (Bio-Idea). In this colorimetric method, the mitochondrial dehydrogenase activity of proliferating cells reduces the MTT salt into purple MTT formazan crystals. After incubation of 10⁴ treated cells/well for 24 h, 10 µL of MTT solution (5 mg/mL) was added to each well and incubated for additional 4 h. The MTT was reduced to blue formazan crystals due to mitochondrial dehydrogenase activity of living cells. At the end of the incubation time, formazan crystals were dissolved by adding 50 µL dimethyl sulfoxide (DMSO) and the plate was placed on the orbital shaker for 20 min. Finally, optical density (OD) at 570-nm wavelength was measured using a spectrophotometer (Bio-Rad, Model 680). The viability rate (%) of cells in different groups was calculated by the following formula: viability rate = (average OD₅₇₀ nm of treated group/average OD₅₇₀ nm of the control group) ×100%.

**Clonogenic cell survival assay**

We used “plating before treatment” protocol to perform clonogenic cell survival assay.39 In this regard, the cells

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Figure 1 The images of GNPs. (A) Transmission electron microscope image, (B) scanning electron microscope image. 
*Abbreviation:* GNPs, gold nanoparticles.
were washed twice with phosphate-buffered saline (PBS), trypsinized, centrifuged, and then counted. A known numbers of cells (100, 200, 400, 1,000, and 2,000 cells, respectively for irradiation dose of 0, 2, 4, 6, and 8 Gy) \(^{16}\) were treated with predefined treatment protocol (Figure 2). The treated cells were incubated for 14 days to allow them to form large colonies. Then, the cells were fixed and stained with 0.4% crystal violet, and the visible colonies with more than 50 cells were counted. The plating efficiency (PE) was determined at IR dose of 0 Gy by the following formula: colony number/plating cell number. The survival fraction (SF) of treatment groups was calculated using the equation: SF = colony number/(plating cell number × PE). The data were fit to linear quadratic model with the equation of SF = \(\exp (-\alpha - \beta D^2)\), and survival curve was estimated using MATLAB software. The dose enhancement factor (DEF) was calculated by dividing of LD\(_{50}\) (50% lethal dose) of irradiation alone group with the combined treatment group.

Statistical analysis

All experiments were performed in replicates of three, and results were reported as mean ± standard error of mean (SEM). To evaluate the cytotoxicity of GNP, the difference between untreated control group and those treated with GNP was assessed by paired t-test. In addition, one-way analysis of variance (ANOVA) was used to compare the differences between the treatment groups. The \(\alpha\) and \(\beta\) parameters of survival curve were calculated with weighted least square regression and SigmaStat statistical software (SPSS Inc., Chicago, IL, USA). In all experiments, the statistical significance was set at \(P \leq 0.05\).

Results

The cytotoxicity of GNP

The cytotoxicity of GNP at a concentration of 0.1 mM on HT-29 and CHO cells was assessed by MTT assay. The SFs of cells after incubation with GNP for 24, 48, and 72 h are presented in Figure 3A and B. The analysis of data was performed with paired t-test and showed no significant cytotoxicity effect on both cell lines (\(P > 0.05\)).

Radiosensitivity of GNP, EP, and GNP-EP

Clonogenic cell survival assay

Figure 4A and B shows the survival curves of HT-29 tumor cells and CHO normal cells that were treated in different groups. In the control group, treatment of HT-29 and CHO cells with irradiation only resulted in LD\(_{50}\) of 3.97 Gy and 4.19 Gy, respectively (Table 1). Adding GNP to culture medium of cells immediately before irradiation decreased the LD\(_{50}\) to 3.37 Gy in HT-29 and 3.63 Gy in CHO cells. In this group, GNP could enhance treatment response by factor of 1.17 and 1.15 in HT-29 and CHO cell lines, respectively. For HT-29 cell line, the LD\(_{50}\) and DEF values, respectively, reached to 2.69 Gy and 1.47 in the group treated with GNP for 24 h before IR. The corresponding values in the CHO cell lines were, respectively, 3.03 Gy and 1.38. For both cell lines, the LD\(_{50}\) values were significantly lower than the control group (\(P < 0.05\)). When the cells were exposed to electric pulse prior to irradiation, LD\(_{50}\) of 2.92 Gy and DEF of 1.36 for HT-29 cells (\(P < 0.05\)) and of 3.27 Gy and 1.28 for CHO cells (\(P < 0.05\)) were observed. In the GNP+EP+IR group, treatment of cells with GNP and EP simultaneously 10 min before irradiation significantly decreased the LD\(_{50}\) values in both cell lines (\(P < 0.05\)). For this treatment protocol, the DEF was 2.61 in HT-29 and 1.92 in CHO cell lines. In the GNP (24 h)+EP+IR group, when the 24 h incubated HT-29 cells with GNP were exposed to electric pulse 10 min prior to irradiation, the LD\(_{50}\) was further reduced to 1.37 Gy in HT-29 cells and to 1.85 Gy in CHO cell lines. Furthermore, in this group, the highest DEF was achieved (2.89 for HT-29 and 2.26 for CHO cell lines). Moreover, the
DEF values demonstrated that synergistic effect was only observed in the HT-29 cells (Table 1).

**MTT assay**

The viability of HT-29 and CHO cells in six different treatment groups was evaluated by MTT assay 24 h after irradiation with 6 MV X-ray at doses of 2 Gy and 4 Gy (Figure 5A and B). In the control group (IR), exposing the cells by 2 Gy irradiation alone yielded a viability of 76.94%±1.2% and 77.54%±0.8% for HT-29 and CHO cell lines, respectively. The treatment of HT-29 and CHO cell lines with 4 Gy irradiation alone reduced the viability to 48.3%±0.18% and 48.67%±0.72%, respectively. No significant reduction in viability was observed when the both cells were received

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**Figure 3** (A) Survival fraction of HT-29 cells incubated with GNPs at a concentration of 0.1 mM for 24, 48, and 72 h compared to nontreated group. None of the groups were statistically significant (P > 0.05). (B) Survival fraction of CHO cells incubated with GNPs at concentration of 0.1 mM for 24, 48, and 72 h compared to nontreated group. None of the groups were statistically significant (P > 0.05).

**Abbreviations:** CHO, Chinese hamster ovary; GNPs, gold nanoparticles.

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**Figure 4** (A) Radiation survival curves of cancerous HT-29 cells treated with six different protocols based on clonogenic assay. (B) Radiation survival curves of CHO cells treated with six different protocols based on clonogenic assay.

**Abbreviations:** IR, ionizing radiation; GNPs, gold nanoparticles; EP, electroporation; SF, survival fraction.
Table 1 Values of α, β, LD_{50}, DEF, and R² values of HT-29 and CHO cells in different groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>α (Gy⁻¹)</th>
<th>β (Gy⁻²)</th>
<th>LD_{50} (Gy)</th>
<th>DEF</th>
<th>R²</th>
</tr>
</thead>
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<tr>
<td></td>
<td>CHO</td>
<td>HT-29</td>
<td>CHO</td>
<td>HT-29</td>
<td>CHO</td>
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<tr>
<td>IR</td>
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<td>0.09±0.013</td>
<td>0.024±0.002</td>
<td>0.021±0.002</td>
<td>4.19</td>
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<td>0.099±0.018</td>
<td>0.14±0.013</td>
<td>0.025±0.003</td>
<td>0.018±0.002</td>
<td>3.63</td>
</tr>
<tr>
<td>GNP (24 h)+IR</td>
<td>0.17±0.017</td>
<td>0.21±0.013</td>
<td>0.01±0.003</td>
<td>0.016±0.002</td>
<td>3.03</td>
</tr>
<tr>
<td>EP+IR</td>
<td>0.145±0.02</td>
<td>0.174±0.024</td>
<td>0.02±0.003</td>
<td>0.021±0.004</td>
<td>3.27</td>
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<tr>
<td>GNP+EP+IR</td>
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<td>0.021±0.004</td>
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<td>GNP (24 h)+EP+IR</td>
<td>0.319±0.02</td>
<td>0.487±0.029</td>
<td>0.028±0.003</td>
<td>0.011±0.005</td>
<td>1.85</td>
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</tbody>
</table>

Note: The values are expressed as mean ± SEM.

Abbreviations: LD_{50}, 50% lethal dose; CHO, Chinese hamster ovary; IR, ionizing radiation; GNP, gold nanoparticles; EP, electroporation; DEF, dose enhancement factor; SEM, standard error of mean.

GNPs immediately prior to irradiation (P>0.05). The viability of HT-29 cells that were exposed to irradiation after 24-h incubation with GNP reached 57.1%±1.1% for 2 Gy and 29.9%±1.7% for 4 Gy irradiation (P<0.05). The treatment of CHO cells with the same protocol resulted in a viability rate of 61.19%±1.2% and 38.95%±0.5% for 2 Gy and 4 Gy irradiation (P<0.05), respectively. By delivering the electric pulse 10 min before IR, the viability rate of 65.91%±0.55% at a dose of 2 Gy and 34.71%±0.93% at a dose of 4 Gy was obtained for HT-29 cells. Almost equal values were observed

Figure 5 (A) The viability of HT-29 cells treated with IR, GNP+IR, GNP (24 h)+IR, EP+IR, GNP+EP+IR, and GNP (24 h)+EP+IR based on MTT assay. *P<0.05. (B) The viability of CHO cells treated with IR, GNP+IR, GNP (24 h)+IR, EP+IR, GNP+EP+IR, and GNP (24 h)+EP+IR based on MTT assay. *P<0.05.

Abbreviations: CHO, Chinese hamster ovary; IR, ionizing radiation; GNP, gold nanoparticles; EP, electroporation.
for CHO cell lines. Indeed, EP could significantly enhance response of HT-29 and CHO cells to irradiation (P<0.05). In the GNPs+EP+IR group, application of both GNPs and EP 10 min before irradiation resulted in a higher radiation response of cells. After delivering of 4 Gy irradiation, only 15.61%±0.26% of HT-29 cells and 52.95%±1.5% of CHO cells were viable (P<0.05). The lowest viability in both radiation doses of 2 Gy (36.80%±1.35% for HT-29 cells and 48.19%±0.84% for CHO cells) and 4 Gy (12.29%±0.72% for HT-29 cells and 19.45%±2.19% for CHO cells) was achieved in GNPs (24 h)+EP+IR group (P<0.05). The results of these tests revealed that concurrent applications of GNPs and EP as radiosensitizer can significantly increase the efficiency of radiation therapy.

Discussion

Radio sensitizing effects of GNPs and EP have been extensively investigated in several in vitro and in vivo studies. However, to our knowledge, there is no published study that investigated the radiosensitizing effects of combined GNPs-EP in any healthy or tumor cell lines. The main objective of this study was to investigate synergistic radiosensitizing effect of combined GNPs-EP to 6 MV X-ray photons. In addition, different combinations of GNPs-EP were investigated to shed more light on the mechanisms of actions of possible radiosensitizing effects.

Our results showed that EP alone could sensitize both HT-29 and CHO cell lines to 6 MV photons with a DEF of 1.36 and 1.28, respectively. Consistent with our result, Kranjc et al.37 reported a DEF of 1.25 for LPB sarcoma cell line. Moreover, West19 demonstrated that EP can enhance effect of 137Cs-γ radiation by a factor of 1.19. Generally, the oxidative jump at electroporated sites of membrane and production of ROS are the probable radiosensitization mechanism of EP.55 Shil et al.43 measured the level of generated ROS induced by EP and reported that the ROS level under the combined EP-irradiation group was significantly higher than irradiation alone group.

In the GNPs+IR group, GNPs did not significantly enhance the effect of IR due to having not enough time for GNPs to enter and accumulate in the target cells. As we know, when irradiation interacts with GNPs, free radicals are produced that further generate ROS. These ROS are very toxic for tumor cells, but have very short diffusion range about 10 nm. Therefore, the accumulation of GNPs in the target cells is essential to induce death in the tumor cells.44 However, in the GNPs+IR group, there was not enough time between treatment of cells with GNPs and IR. However, when EP is added to the protocol of GNPs+IR group (GNPs+EP+IR), DEFs of 2.61 and 1.92 were observed in HT-29 and CHO cells, respectively. The observed difference between DEF values of GNPs+IR and GNPs+EP+IR groups can be attributed to the act of EP as a GNPs delivery system in GNPs+EP+IR group.

Incubation of the cells with GNPs 24 h prior to IR (GNPs (24 h)+IR) resulted in the DEF of 1.47 and 1.38, respectively, for HT-29 and CHO cells. However, these values reached to 2.89 and 2.26 in HT-29 and CHO, respectively, by introducing EP in this protocol 10 min before IR (GNPs (24 h)+EP+IR). In this group, EP showed no delivery system role. Indeed, the GNPs that could not enter to cells during 24 h were eliminated during washing cells with PBS, trypsination, and centrifuging processes prior to EP, and thus this radiosensitizing effect is related to intrinsic radiosensitivity of EP due to ROS generation.

Our findings support the previous studies that have investigated the radiosensitizing effect of GNPs alone. Different mechanisms have been proposed in these studies for radiosensitivity induced by GNPs. The cell cycle regulation or accumulation of DU-145 prostate cancer cells in G2/M phase as a most radiosensitive phase of cell cycle due to activation of CDK kinase was reported by Roa et al.40 Wang et al.40 demonstrated that Glu-GNPs increased apoptosis by overexpression of Bax and caspase 3 and underexpression of Bcl-2 proteins. Other probable mechanisms are increasing of ROS production and DNA double strand break, and these have been proposed by Geng et al.46 and Chithrani et al.7 respectively.

Radio sensitizing effect of GNPs is dependent on three important factors: size, concentration of GNPs, and energy of the ionization radiation source.47 The effects of size and concentration of PEG-coated GNPs have been comprehensively investigated by Zhang et al.48 Their in vitro and in vivo studies revealed that for all sizes of 4.8, 12.1, 27.3, and 46.6 nm, the concentration of 0.1 mM was safe and nontoxic. The strongest radiosensitizing effect with SER of 2.07 was obtained with 12.1 nm GNPs at concentration of 0.1 mM. Similarly, in vivo radiotherapy demonstrated that all sizes of GNPs can enhance the effect of 5 Gy radiation.45 Similarly, we used 12–15 nm GNPs at concentration of 0.1 mM, and the results of MTT assay revealed that this dose of GNPs is safe for all incubation times of 24, 48, and 72 h.

Several studies have been conducted on radio sensitivity effect of GNPs with KV radiations due to a Z4 relationship between photoelectric cross section and atomic number (Z).49,50 However, Jain et al.51 comprehensively evaluated the impact of type and energy of radiation (kV and MV photons and MV electrons) on GNPs radiosensitizing. They reported...
that 1.9 nm GNPs could significantly sensitize MDA-MB-231 breast cancer cells by a factor of 1.41, 1.29, 1.16, and 1.35 using 160 kVp, 6 MV, and 15 MV X-ray photons, and 16 MeV electrons, respectively. Furthermore, Wang et al reported that treatment of A549 cells with 13 nm Glu-GNPs and 6 MV photons resulted in a DEF of 1.49. According to these results and because of the extensive uses of MV photons to treat deep tumors as well as spare skin of patients in clinic, we decided to use 6 MV photons for irradiation.

In addition, the cell type is another factor that can influence the outcome of treatment with GNPs. During the recent years, the radiosensitizing feature of GNPs has been used to treat several cell lines such as prostate, breast, lung, and colorectal cancer cell lines. Arab-Bafrani et al observed that the response of HT-29 colorectal cancer cell line to 9 MV photons was increased by a factor of 1.4 using GNPs. Similarly, in our study using only GNPs 24 h prior to irradiation, a DEF of 1.47 was observed. However, the GNPs (24 h)+EP+IR protocol resulted in a DEF of 2.89 in the HT-29 cancer cell line. In addition, the synergistic effect was only observed in the HT-29 cell.

**Conclusion**

In conclusion, to our knowledge, this study was the first to use both GNPs and EP simultaneously to sensitize cells to 6 MV radiations. The most radiosensitizing effect was achieved with GNPs (24 h)+EP+IR protocol in HT-29 cell with intermediate intrinsic radiosensitivity. Therefore, this protocol has a potential to sensitize less radiosensitive tumor cells, and thus there is need for more in vivo studies to translate this approach into the clinic.

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**Author contributions**

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

**Disclosure**

The authors report no conflicts of interest in this work.

**References**