



Gold-coated iron oxide nanoparticles trigger apoptosis in the process of thermo-radiotherapy of U87-MG human glioma cells

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Abstract

Recently, gold-coated iron oxide nanoparticles (Au@IONPs) have received a great deal of attention in cancer therapy. In this *in vitro* study we aimed to investigate the anti-cancer effects of Au@IONPs core–shell nanoparticles when applied in thermo-radiotherapy. Moreover, we investigated the level of apoptosis induced in U87-MG human glioma cells after receiving a combinatorial treatment regimen (Au@IONPs + hyperthermia + radiotherapy). Firstly, the Au@IONPs nano-complex was prepared and characterized. Cytotoxicity of the nanoparticles (various concentrations; 4 h incubation time) was investigated on U87-MG cells and finally the concentrations of 10 and 15 $\mu\text{g}/\text{mL}$ were selected for further studies. After incubation of the cells with nanoparticles, they received hyperthermia (43 °C; 1 h) and then were immediately exposed to 6 MV X-ray (2 and 4 Gy). Following the treatments, MTT assay was used to analyze cell viability and flow cytometry was used to determine the level of apoptosis in each treatment group. The results revealed that nanoparticles have no significant cytotoxicity at concentrations lower than 10 $\mu\text{g}/\text{mL}$. Also, we observed that nanoparticles are able to enhance the cytotoxic effect of hyperthermia and radiation. The major mode of cell death was apoptosis when nanoparticles, hyperthermia and radiation were concomitantly applied to cancer cells. In conclusion, Au@IONP nanoparticle can be considered as a good thermo-radio-sensitizer which triggers significant levels of apoptosis in cancer therapy.

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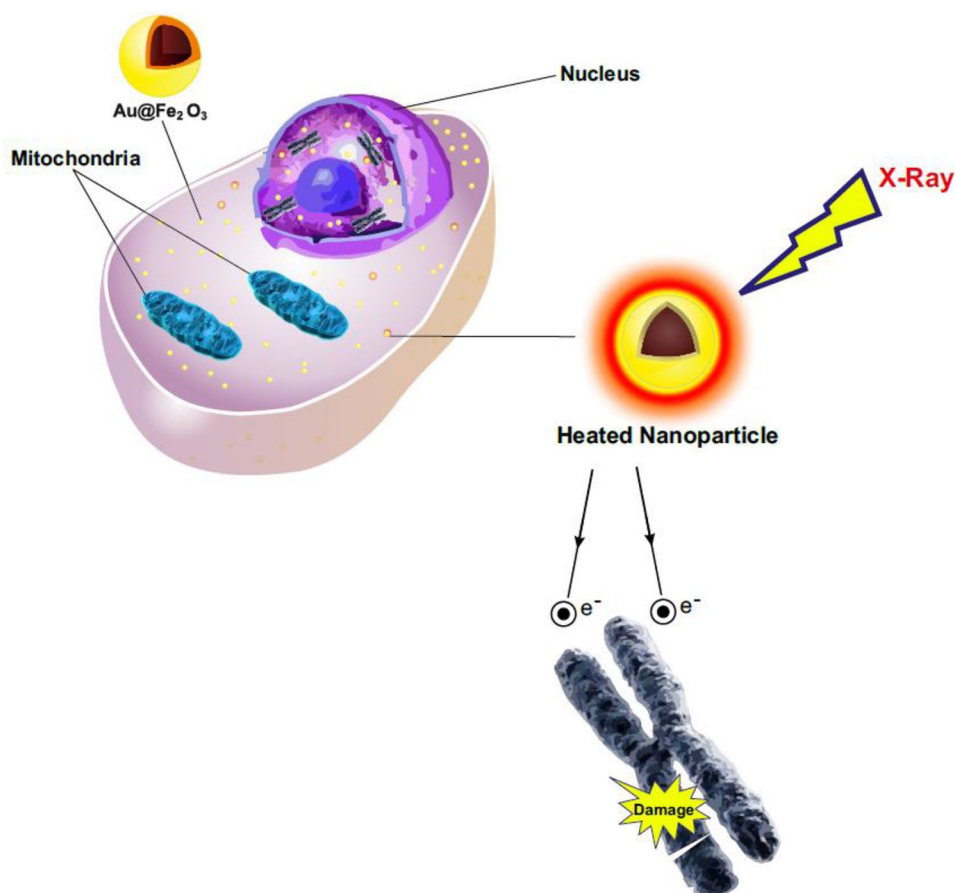
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Graphical abstract



In this *in vitro* study, we report the anti-cancer effects of gold-coated iron oxide nanoparticles (Au@IONPs) when applied in thermo-radiotherapy.

Keywords Cancer · Core–shell nanoparticles · Radiotherapy · Hyperthermia · Apoptosis

Introduction

Glioblastoma is the most common and malignant type of brain tumors which is characterized by outspread invasion through the brain. Unrestricted cellular multiplications, durable angiogenesis, gentle necrosis, resistance against apoptosis and conventional treatment, molecular instability and normal brain tissue loss are the most important signs of glioblastoma tumors (Furnari et al. 2007). Surgery, chemotherapy, and radiation therapy are the main treatment modalities of brain tumors. In spite of broad advancement in these fields, the survival of patients is poor (Mahdavi et al. 2016; Sun et al. 2013). In the process of treatment, each of the mentioned methods has particular restrictions. Brain tumors are often inaccessible and sometimes are not available for neurosurgeons during operation (Hehr et al. 2003). The most important limitation that prevents a successful use of

chemotherapy is intolerable side effects (due to systemic distribution and high toxicity of drugs) imposed to the patients (Sudhakar 2009). Considering the benefits of radiotherapy, particularly for brain tumors, this method also has serious complications. One of the most important complications is that the recommended high doses of radiotherapy are harmful for the healthy brain tissue around the tumor (Witteles 2011; Manjili et al. 2014). These restrictions encourage investigations of new methods for cancer therapy (Sun et al. 2013; Zhou et al. 2011).

In addition to all conventional treatment modality mentioned above, hyperthermia is considered as another cancer treatment method (Beik et al. 2016a, b). In hyperthermia, the temperature of the whole body or a part of the body is raised to 43–48 °C which causes destruction of intracellular structures and changes in cytoskeleton organization, cytoplasmic membranes and proteins. These damages lead

to cancer cell death. Another advantage of hyperthermia is its efficient effects when combined with radiotherapy. Heating the tissue enhances blood flow and, consequently, increases tumor tissue oxygen level that can strengthen radiotherapy response. Therefore, hyperthermia can be considered as a method of radiosensitizing cancer cells (Chicheł et al. 2007; Hildebrandt et al. 2002). In spite of all benefits mentioned for hyperthermia, it suffers from non-selectivity (Samadian et al. 2016). This means that both normal and cancer cells absorb the heat, limiting the therapeutic benefit (Verma et al. 2014).

With the advent of nanotechnology and considering the profound effects of various nanomaterials, new cancer treatment methods are under investigation (Bañobre-López et al. 2013; Cabuy 2011; Van der Zee 2002; Beik et al. 2017). The gold nanoparticle (AuNP) has a high atomic number (Z) and, as a result, a high photon absorption cross-section (Li et al. 2016). The nanoparticle can enhance the biological effects of radiation in cancer cells, such as increasing the level of apoptosis. Since they have high biocompatibility, AuNPs are a good choice to be applied in various biomedical fields, particularly in cancer hyperthermia and radiotherapy (Khoshgard et al. 2014). Magnetic nanoparticles, such as iron oxide nanoparticle (IONP), are considered as another class of nanoparticles with a high potential in various fields of biomedicine (for example in cancer diagnosis and therapy) (Eyvazzadeh et al. 2017). IONPs can play a role as a contrast agent in magnetic resonance imaging contributing to better and more sensitive cancer diagnosis protocols (Manjili et al. 2014). Nanoparticles with high magnetic power, high heat absorption, low toxicity and large amounts of accumulation in the tumor are helpful and have been recently introduced into the area of cancer hyperthermia and radiotherapy (Verma et al. 2014; Mirrahimi et al. 2017; Silva et al. 2011). There is a possibility that in the presence of magnetic nanoparticles, the cell absorbs more heat increasing oxidative stress, ultimately augmenting cell death (Soares et al. 2012; Esmaelbeygi et al. 2015; Cabada et al. 2012). Moreover, significant effects of IONPs on cancer cell thermosensitizing and radiosensitizing have been recently reported (Esmaelbeygi et al. 2015).

A major problem in treating brain tumors using nanotechnology-based hyperthermia or radiotherapy is the blood–brain barrier (BBB) that does not allow the therapeutic agents to enter the brain tissue. However, there is some evidence demonstrating that nanotechnology-assisted cancer therapy overcomes the BBB by several mechanisms: (1) brain tumors cause serious damage to the BBB and disrupt its integrity, (2) radiation can also destruct the BBB enabling nanoparticles to cross the BBB, (3) some nanoparticles, such as magnetic nanoparticles, can cross the BBB through leaky vessels of brain tumors and remain in the lesion because of

a physiologic phenomenon known as enhanced permeability and retention (EPR) (Maeda et al. 2009; Joh et al. 2013).

Considering the potentials of gold and magnetic nanoparticles outlined above, it is interesting to investigate the biological effects of both AuNPs and IONPs. Hoopes et al. have recently studied the effect of IONPs and AuNPs in combination with hyperthermia and radiotherapy. The results of their studies confirm that the application of these nanoparticles induce better responses to radiotherapy and hyperthermia (Hoopes et al. 2007; Hu et al. 2017). In addition, the Au@IONPs core–shell nanoparticles have received a great deal of attention in cancer therapy (Miyake et al. 2008). The aim of the present *in vitro* study was to investigate the anti-cancer effect of Au@IONPs nanoparticles in combination with radiotherapy and hyperthermia using U87-MG human glioma cells. Given the diverse biological consequences of inducing necrosis versus apoptosis, we specifically focused on the process of apoptosis induction.

Materials and methods

Materials

Au@Fe₂O₃ core–shell nanoparticles (NP, code: NB-CS-30) were purchased from Nanobon Company (Tehran, Iran). A brief explanation of the nanoparticle synthesis procedure is given in the next section. The U87-MG cell line was received from the Pasteur institute (Tehran, Iran). Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were purchased from GIBCO (Invitrogen, Germany). Trypsin ethylene diamine tetra acetic acid (EDTA) and penicillin–streptomycin solution and dimethyl sulfoxide (DMSO) and MTT(3-(Hehr et al. 2003)-2, 5-diphenyltetrazolium bromide) were purchased from Sigma (St Louis, MO, USA). Flow cytometry kit (Annexin V/PI) was purchased from eBioscience company (San Diego, CA, USA).

Synthesis of Au@IONPs nanoparticles

Au@Fe₂O₃ core–shell NPs were prepared through the oxidation of Fe₃O₄ NPs in the aqueous phase (Kang et al. 1996). Briefly, 2.6 g of FeCl₃ and 1.0 g of FeCl₂ were dissolved in HCl solution (12 M) and 12.5 mL of H₂O. The solution was introduced into a NaOH solution through dropwise addition (125.0 mL, 1.5 M) with vigorous stirring. The generated black precipitate was collected using a magnet and the supernatant was removed from the precipitate through decantation. After being washed three times using 50 mL of water, HCl solution (250 mL, 0.01 M) was added to neutralize the anionic charges of the precipitate; the resulting Fe₃O₄ NPs were obtained after washing with water. The freshly prepared Fe₃O₄ NPs were further dissolved in HNO₃ (0.01 M)

and heated under stirring at 100 °C for 1 h to completely oxidize the Fe₃O₄ NPs to Fe₂O₃ NPs. The solution was then cooled to room temperature and washed twice using water, followed by centrifugation at 10,000 RPM for 15 min. Au@Fe₂O₃ core–shell NPs were synthesized through the deposition of Au on Fe₂O₃ NPs using the modified Lyon's iterative hydroxylamine-seeding procedure (Lyon et al. 2004). First, Fe₂O₃ NPs (1.0 mL, 1.1 mM) were mixed with sodium citrate (1.0 mL, 0.1 M) for 10 min, followed by dilution to 20 mL using H₂O. Subsequently, 0.1 mL of NH₂OH·HCl solution and 1.0 mL of HAuCl₄ solution were added dropwise under stirring for a total of 50 min. The addition of NH₂OH·HCl and HAuCl₄ was repeated twice more. The solution changed to garnet after three iterative additions.

Characterization of nanoparticles

The morphology and shape of Au@IONPs were determined using transition electron microscopy at an accelerating voltage of 200 KV and a scanning electron microscopy at 26 KV (EM3200 model, KYKY company, China). The hydrodynamic size and zeta potential distributions of nanoparticles were investigated by a Nano-ZS 90 Nanosizer (Malvern Instruments Ltd., Worcestershire, UK).

Cell culture

The human glioblastoma cell line U87-MG was used in this *in vitro* study. U87-MG cells were cultured in high-glucose DMEM containing 10% FBS and 1% penicillin/streptomycin. Cells were kept in a 37 °C incubator with 5% CO₂ and the cells were sub-cultured twice a week upon reaching approximately 80% confluency.

Nanoparticle cytotoxicity and cellular uptake assay

To test the cytotoxicity of core–shell nanocomplex, the viability of the cells incubated with various concentrations of the nanocomplex was evaluated using the MTT tetrazolium assay, which measures the ability of metabolically active mitochondria in live cells to reduce a colorless tetrazolium compound to a blue formazan product. To perform this assay, we seeded 1×10^4 U87-MG cells into each well of a 96-well microplate and after 24 h incubation, cells were incubated with various concentrations of nanocomplex (0–30 µg/mL) for 4 h. Then, the nanocomplex was removed and cells were washed with PBS. MTT assay was performed 24 h post-treatment. After adding 100 µl of FBS-free culture medium, 10 µl of MTT dye (at a concentration of 5 mg/mL) was also added to each well, and the plate was incubated for 4 h. Then, MTT-containing medium was removed and replaced with DMSO (10 µl per well) to dissolve the formazan crystals and kept in the dark

at room temperature for 15 min. Finally, the absorbance of the dissolved formazan was read at a wavelength of 570 nm by an ELISA reader (DYNEX MRX, USA) using a reference wavelength of 630 nm. The relative survival of U87-MG cells was expressed as the absorbance of the treated sample/absorbance of the control group. The optical density (OD) of dissolved formazan is directly proportional to the number of living cells. All experiments were done in triplicate. In our experiments, we designed control groups beside the treated groups and the percentage of viable cells was calculated by the following formula:

$$\text{Cell viability(\%)} = \frac{[\text{OD}_{\text{sample}} - \text{OD}_{\text{medium}}]/(\text{OD}_{\text{control}} - \text{OD}_{\text{medium}})] \times 100.}$$

After incubation of U87-MG cells with nanoparticles for 4 h, ICP-MS and TEM analyses were performed to determine the uptake of the nanoparticles by cancer cells. The cells that had been exposed to nanoparticles at a concentration of 15 µg/mL and incubated for 4 h were processed for TEM analysis. Samples were fixed with 2.5% glutaraldehyde, pH 7.2. After primary fixation, the samples were intensively rinsed with 0.1M phosphate buffer (pH 7.2), then post-fixed with 1% OsO₄ for 2 h and finally dehydrated in an acetone solution. The resulting dried specimens were embedded in Epoxy Resin (TAAB 812) which was polymerized at 60 °C for 48. Thin, 70 nm sections were sliced for TEM analysis using an ultramicrotome. Sections were transferred on 200 mesh grids and stained with heavy metals (uranyl acetate and lead citrate). Stained samples were visualized under TEM LEO 906 (80 kV).

For ICP-MS studies, 4×10^5 cells incubated with nanoparticles at a concentration of 15 µg/mL for 4 h and were analyzed using ICP-MS ELAN DRC-e spectrometer (PerkinElmer SCIEX, Concord, Ontario, Canada). The incubated cells were digested in 3 mL of aqua regia. Following digestion, samples were diluted to 10 mL with reagent water and analysis was performed for gold.

Hyperthermia and radiation therapy

Hyperthermia was applied to the cells using a water bath hyperthermia system. In this treatment method, U87-MG cells were immersed in a water bath and maintained at a constant temperature of 43 °C for 1 h.

For analyzing the impact of radiation, the U87-MG cells were irradiated by a medical LINAC accelerator (Siemens, Germany, running at 6 MV) with doses of 2 and 4 Gy. Cells received the exposure from posterior at a distance of 100 cm from the bottom of the plate and radiation field size was 40 × 40 cm.

Combined effect of hyperthermia and radiotherapy

To study the combinatorial effects of hyperthermia and radiotherapy (with or without nanoparticles), the U87-MG cells were cultured in 96-well microplates and maintained in an incubator for 24 h. After removing the culture medium, nanoparticles at concentrations of 10 and 15 $\mu\text{g}/\text{mL}$ were added to each well and then maintained in an incubator for 4 h. Then, U87-MG cells received hyperthermia (at 43 $^{\circ}\text{C}$) for 1 h. After hyperthermia, the cells were irradiated with 2 and 4 Gy of X-ray. The average time between hyperthermia and radiotherapy was 10 min. After a combined treatment, the cells were kept in an incubator for 24 h and analyzed for viability by the MTT assay.

Flow cytometry

The level of apoptosis was measured using an annexin V–fluorescein isothiocyanate (FITC) apoptosis detection kit, as described in the manufacturer’s instruction (eBioscience, USA). In brief, cells were plated in 6-well plates at a concentration of 5×10^5 cells/mL and the plates were kept in an incubator for 24 h. Then, various treatment modalities were applied. After 24 h, the cells were harvested and centrifuged at 300 g for 5 min. Then, the cells were washed once with PBS and once with $1 \times$ binding buffer. After that, cells were suspended in $1 \times$ binding buffer and 5 μl of fluorochrome-conjugated annexin V was added to 100 μl of cell suspension and incubated for 15 min in dark at room temperature. Cells were washed with 2 mL of binding buffer and suspended in 200 μl of $1 \times$ binding buffer. Finally, 5 μl of propidium iodide

(PI) staining solution was added to 200 μl of cell suspensions and assessed by a flow cytometer (BD FACSCantoII, USA).

Statistical analysis

Statistical analysis was performed using the SPSS software (version 11). One-way analysis of variance (ANOVA) was used to evaluate the significance of results. A value of $p < 0.05$ was considered as statistically significant.

Results

Nanoparticles: characteristics and cell uptake

The size, shape and morphology of the prepared Au@IONPs were investigated by TEM and SEM. Images of nanoparticles are shown in Fig. 1. The nanoparticles were spherical in shape and the gold shell on iron oxide core is evident. Figure 2 shows that the hydrodynamic size distribution of nanoparticles ranged from 20 to 50 nm. Zeta potential is an indicator of the surface charge of nanoparticles and it was estimated as 17.9 mV (Fig. 3). To confirm that the nanoparticles were efficiently taken up by the cells, TEM visualization was conducted. The result confirmed the presence of particles in the cell cytoplasm and mitochondria (Fig. 4). ICP-MS studies also confirmed the internalization of nanoparticles (0.1 ng Au per cell).

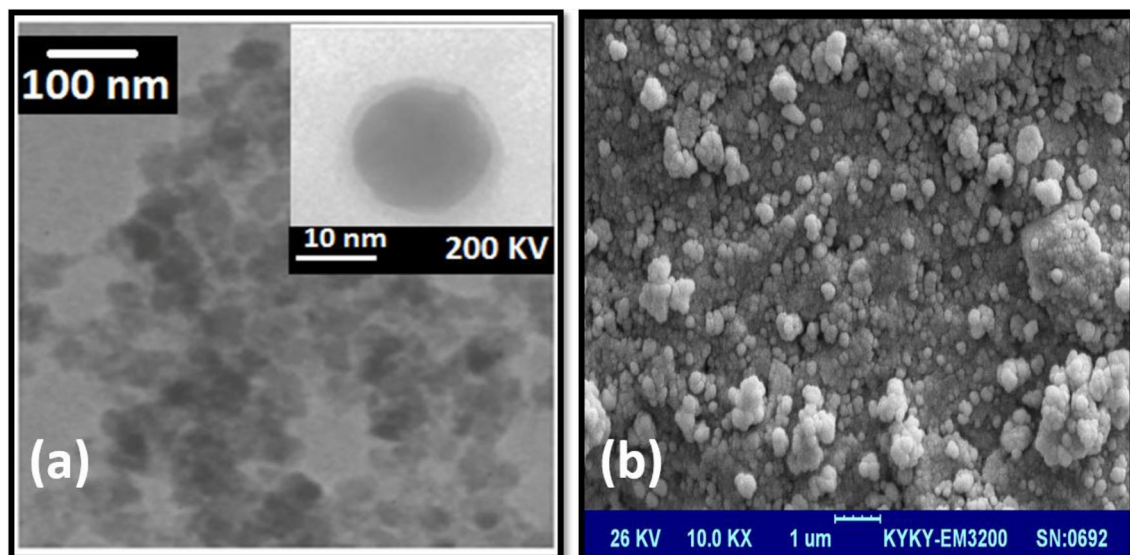


Fig. 1 **a** Transmission electron microscope (TEM) image and **b** scanning electron microscope (SEM) image of the synthesized Au@IONPs nanocomplex

Fig. 2 Hydrodynamic size distribution of the synthesized Au@IONPs nanocomplex

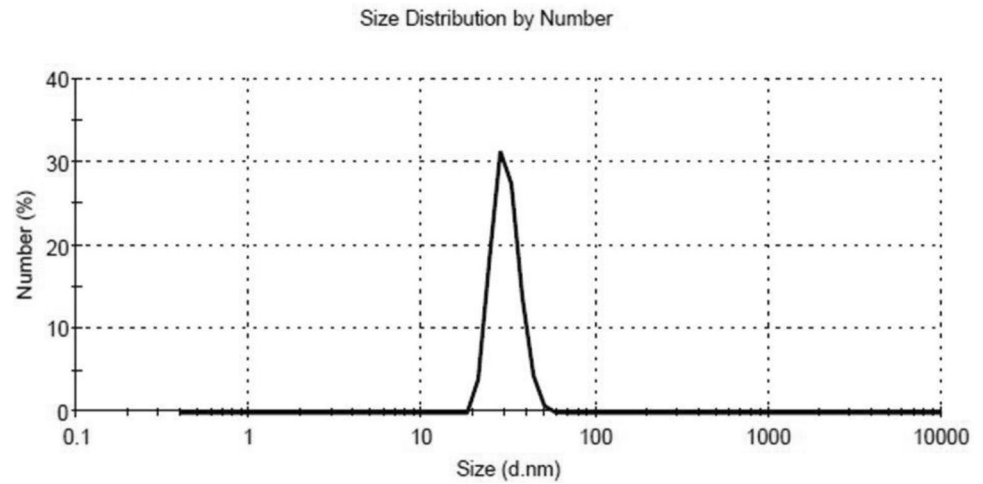


Fig. 3 Zeta potential distribution of the synthesized Au@IONPs nanocomplex

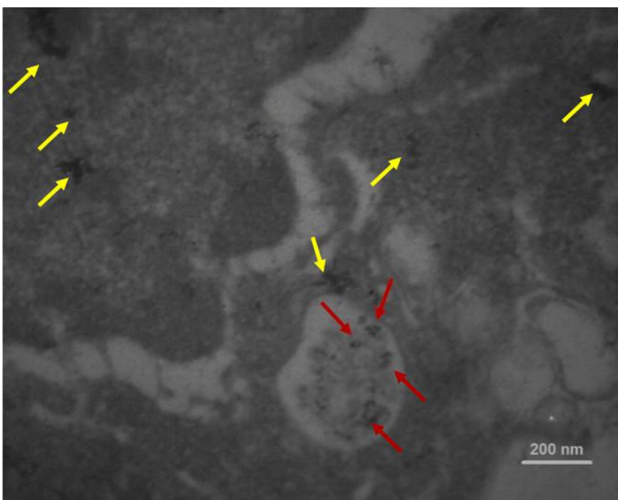
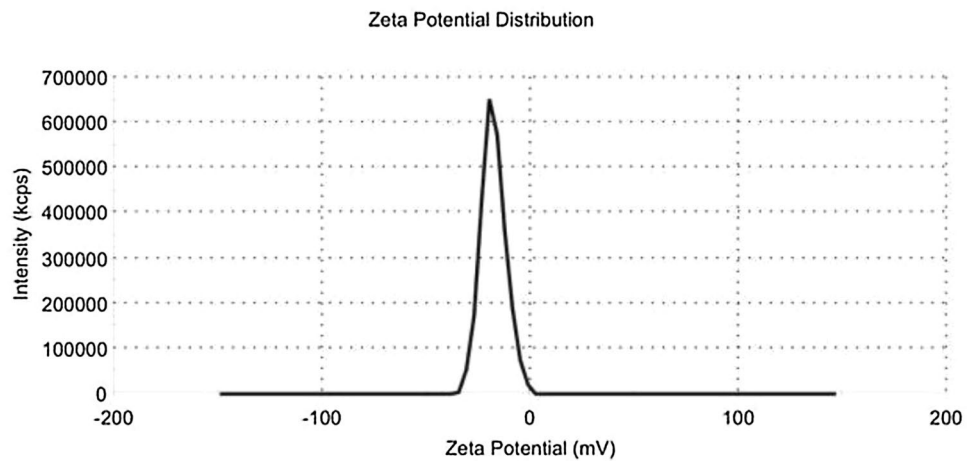


Fig. 4 In vitro cellular uptake assay for the synthesized Au@IONPs nanocomplex. Yellow arrows show nanoparticles inside the cytoplasm and red arrows indicate nanoparticles inside mitochondria

Cytotoxicity of nanoparticles

Following a 4-h incubation of cells with different concentrations of nanoparticles (0–30 $\mu\text{g}/\text{mL}$), cell viability was evaluated 24 h later using the MTT assay. As shown in Fig. 5, Au@IONPs at concentrations below 10 $\mu\text{g}/\text{mL}$ had no significant effect on cell viability, but higher concentrations considerably decreased the viability of U87-MG cells.

Thermosensitizing and radiosensitizing effect of nanoparticles

In vitro thermal effects of nanoparticles were investigated as described in the section “[Hyperthermia and radiation therapy](#)”. Moreover, to assess the radiosensitizing effect of nanoparticles, cells were incubated with nanoparticle concentrations of 10 and 15 $\mu\text{g}/\text{mL}$ for 4 h and then exposed

Fig. 5 The viability of U87-MG cells treated with different concentrations of Au@IONPs nanocomplex (incubation time: 4 h). Error bars show means \pm SD; * p < 0.05 and ** p < 0.001 compared to control group

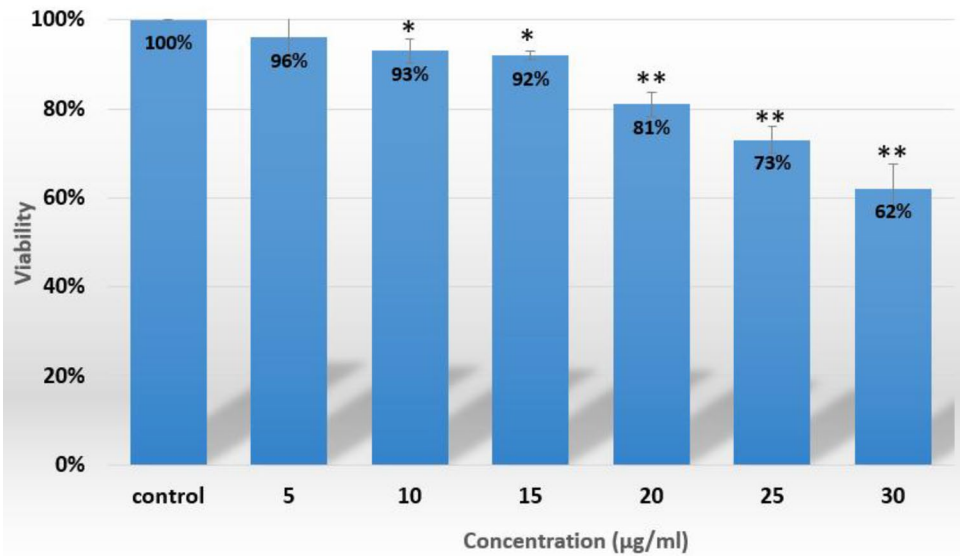


Fig. 6 The cell viability following hyperthermia (HT) in the presence of various concentrations Au@IONPs nanocomplex (incubation time: 4 h). Error bars show means \pm SD, * p < 0.05 and ** p < 0.001 compared to HT group

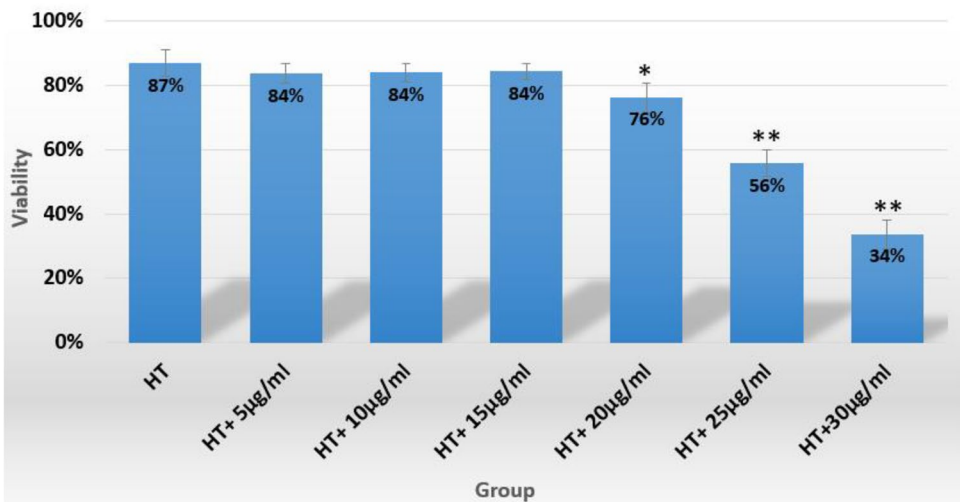
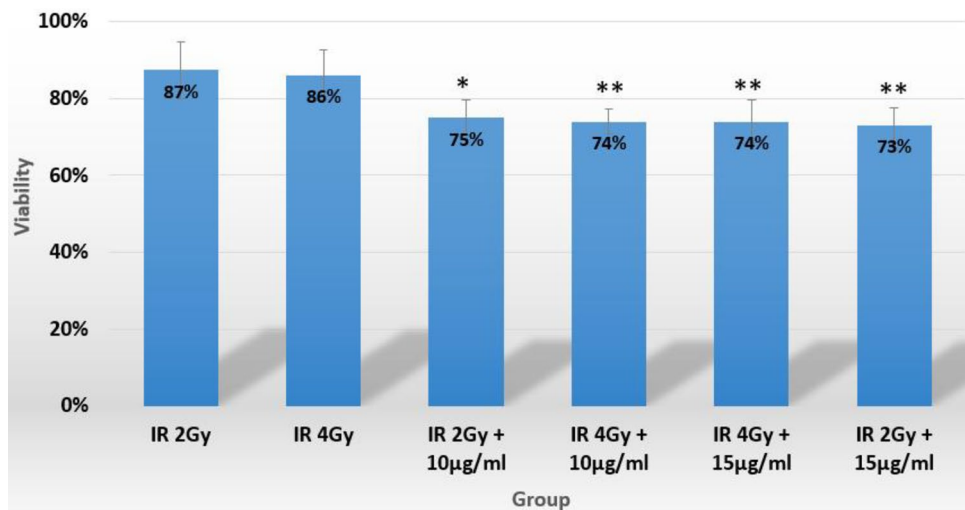


Fig. 7 The viability of U87-MG cells incubated with 10 and 15 µg/mL of Au@IONPs for 4 h and then exposed to 2 and 4 Gy doses of ionizing radiation (IR; 6MV X-ray). Error bars show means \pm SD, * p < 0.05 and ** p < 0.001 compared to IR-2Gy group



to 2 and 4 Gy of 6 MV X-ray radiation. Cell viability was analyzed after 24 h by the MTT assay. As seen in Fig. 6, nanoparticles potentiated the effect of hyperthermia in a dose-dependent manner. Figure 7 shows the impact of nanoparticles on the viability of cells exposed to ionizing radiation. The combination of radiation and nanoparticles induced a modest inhibition of cell growth. For example, when cells were exposed to 2 Gy alone, cell viability was 87%, while it was 75% and 73% for cells incubated with 10 and 15 $\mu\text{g}/\text{mL}$ of Au@IONPs, respectively.

Combination of hyperthermia, radiation and nanoparticles

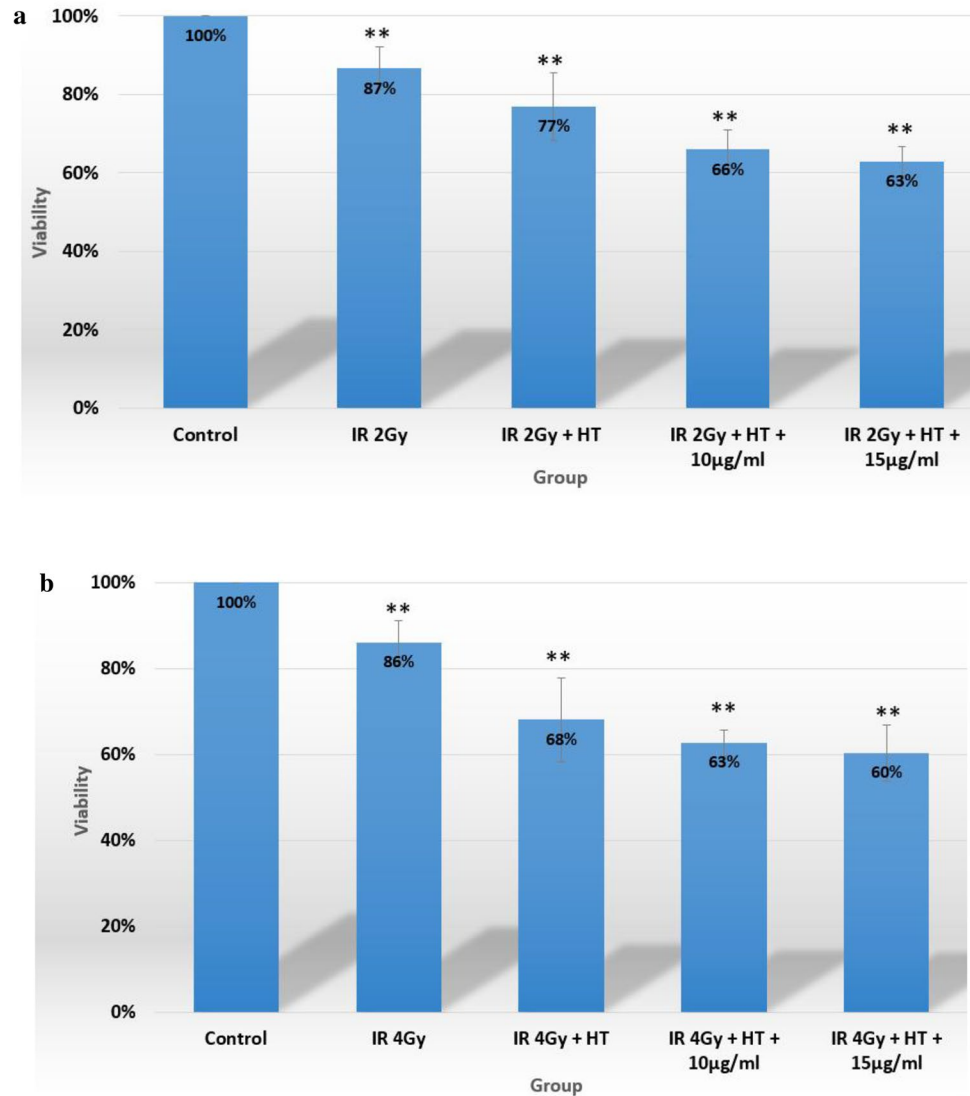
The details of this experiment are described in section “Combined effect of hyperthermia and radiotherapy”. 24 h after the combined treatment the MTT assay was performed and the results are shown in Fig. 8. In comparison

to hypothermia and radiation alone, cell viability decreased when the cells were exposed to hyperthermia and radiation in the presence of nanoparticles.

Apoptosis analysis

One of the most important types of cell death is apoptosis. We, therefore, quantified apoptosis induced by each treatment group. Only the nanoparticle concentration of 15 $\mu\text{g}/\text{mL}$ was applied and apoptosis was analyzed by flow cytometry. The results are shown in Figs. 9 and 10. Right lower and upper quadrants of the flow cytometry diagrams show early and late apoptosis, respectively. The sum of the two apoptosis types is presented in Fig. 11 as total apoptosis. No apoptosis was seen in control cells. The combination of hyperthermia and 2 and 4 Gy of radiation induced 18% and 23% apoptotic cells, respectively. 15 $\mu\text{g}/\text{mL}$ of nanoparticles increased these values to 39% and 42%.

Fig. 8 Viability of U87-MG cells received combination therapy of Au@IONPs (incubated for 4 h), hyperthermia (HT) and ionizing radiation (IR); **a** for 2 Gy and **b** for 4 Gy. Error bars show means \pm SD and $**p < 0.001$ compared to control group



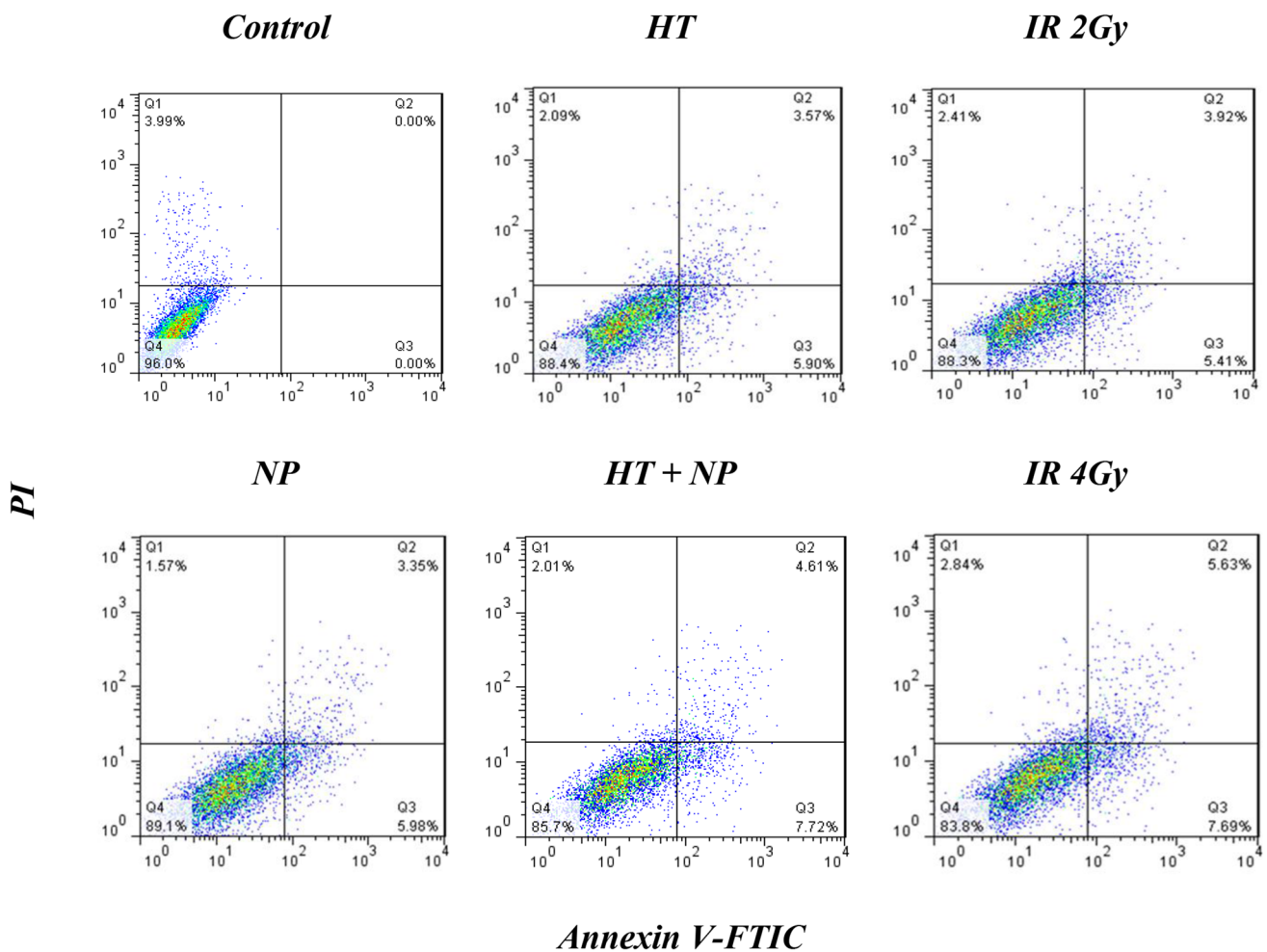


Fig. 9 Flow cytometry analysis to determine the death type of cancer cells after receiving Au@IONPs (15 $\mu\text{g}/\text{mL}$, 4 h), hyperthermia (HT) and ionizing radiation (IR) alone. In addition, the results obtained for (HT+NP) treatment is presented

Discussion

Gold-coated iron oxide nanoparticles (Au@IONPs) have a high potential in biomedical research areas such as cancer diagnosis and therapy (Mahdavi et al. 2016). In this study, we have prepared the Au@IONP nanocomplex and examined its properties as a thermo-radio-sensitizer in therapy of gliomas. The nanocomplex is made of iron oxide nanoparticles which is advantageous for enhancing hyperthermia effects and has a gold layer in its structure which enhances the radiotherapy efficacy (Ban et al. 2005; Park et al. 2007).

We observed an inverse correlation between the dose of the Au@IONP nanoparticles and cell viability. A similar result was reported by Mateo et al. who studied the effect of AuNPs on HL-60 and HepG2 cells (Mateo et al. 2014). The reason of cell death induced by nanoparticles is most probably due to the induction of reactive oxygen species (ROS) leading to apoptosis (Wahab et al. 2014; Gopinath et al. 2010; Butterworth et al. 2010).

Moreover, we observed that Au@IONPs can act as a potent radiosensitizer for megavoltage X-rays as used in external beam radiotherapy. The mechanism of the radiosensitizing effect strongly depends on the X-ray energy. In orthovoltage radiotherapy, the major effects of nanoparticles are due to the photoelectric phenomenon. In megavoltage radiotherapy, nanoparticles enhance the radiotherapy response through chemical or biological mechanisms such as the formation of ROS, induction of DNA damage and DNA repair inhibition (Her et al. 2015; Butterworth et al. 2013; Sicard-Roselli et al. 2014). Yi et al. investigated the radiosensitizing effect of PEGylated Au@MnO₂ nanoparticles in combination with megavoltage X-rays (Yi et al. 2016). They demonstrated that the level of DNA damage was highest in the treatment group receiving both Au@MnO₂ nanoparticles and radiation. In another study, Wang et al. tested thioglucose-AuNP as a radiation enhancer in the breast cancer cell line MDA-MB-231. Their results showed that the nanoparticles influence cell cycle progression and sensitize the cells to

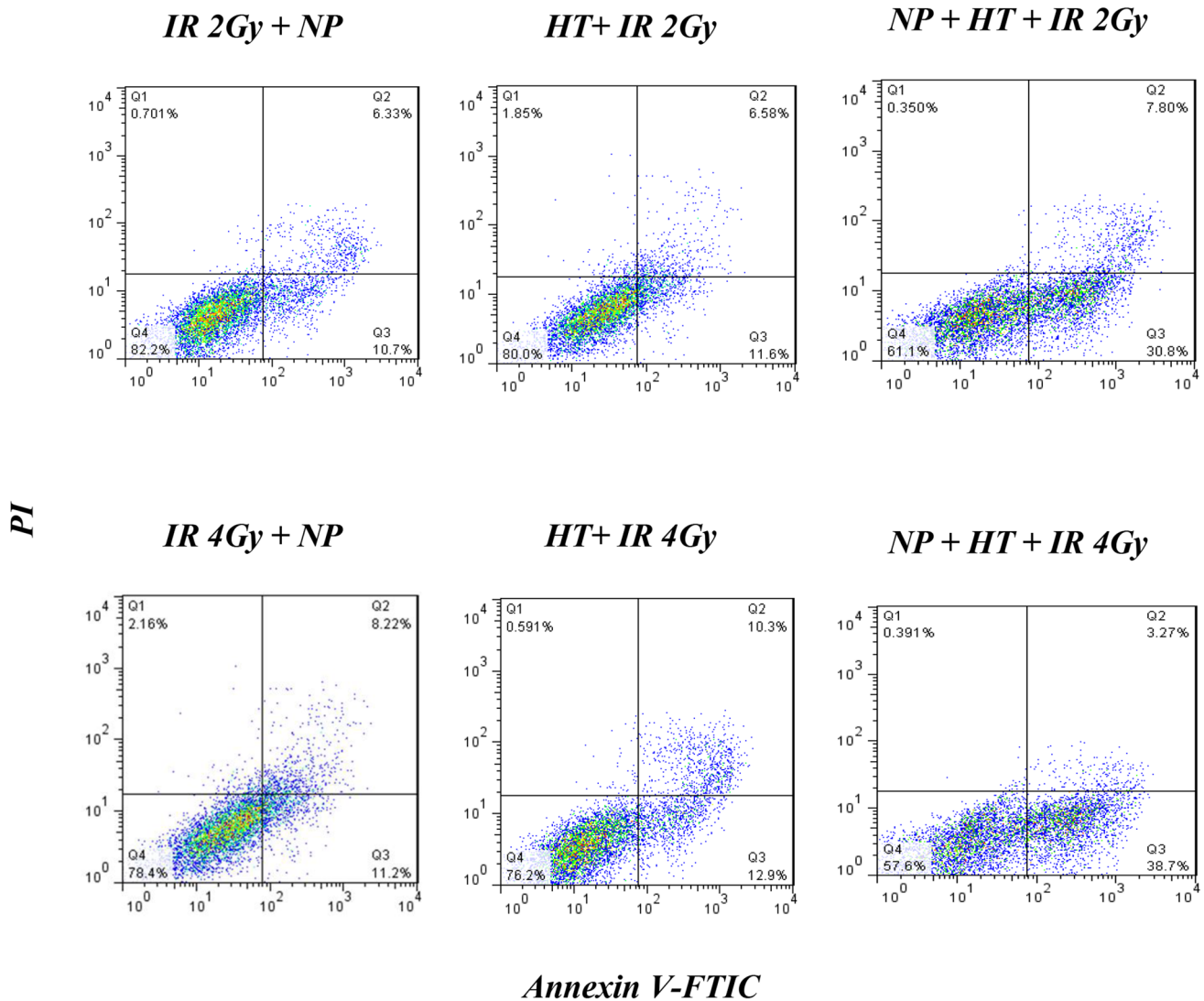


Fig. 10 Flow cytometry analysis to determine the death type of cancer cells after receiving various combinations of Au@IONPs (15 $\mu\text{g}/\text{mL}$, 4 h), hyperthermia (HT) and ionizing radiation (IR)

ionizing radiation so that a significant reduction in survival fraction can be achieved (Wang et al. 2015). In addition, McMahon et al. demonstrated that heavy atom nanoparticles potentiate the effect of radiation by increasing the level of radiation-induced DNA damage (McMahon et al. 2011).

To achieve a better efficacy in cancer treatment, we tested the impact of Au@IONPs on the combination of hyperthermia and radiotherapy. Au@IONPs decreased the viability of cells exposed to both factors beyond the effect expected by assuming additivity (Table 1). To mimic the scenario of radiotherapy, hyperthermia was applied first, then followed by radiation. According to other studies, heat increases the blood

flow so that more oxygen can arrive to the tumor, reducing hypoxia. A reduction in the number of hypoxic cells increases the sensitivity of the tumor. As a result, hyperthermia can help to kill radio-resistant cells and in this way plays a critical role in increasing the efficacy of cancer therapy (Hildebrandt et al. 2002; Ma et al. 2016; Thapa et al. 2015).

We used flow cytometry to analyze the percentage of necrotic and apoptotic cells and found that Au@IONPs increased the apoptosis level in cells exposed to a combination of hyperthermia and radiation. Au@IONPs had no impact on the effect of hypothermia alone but potentiated

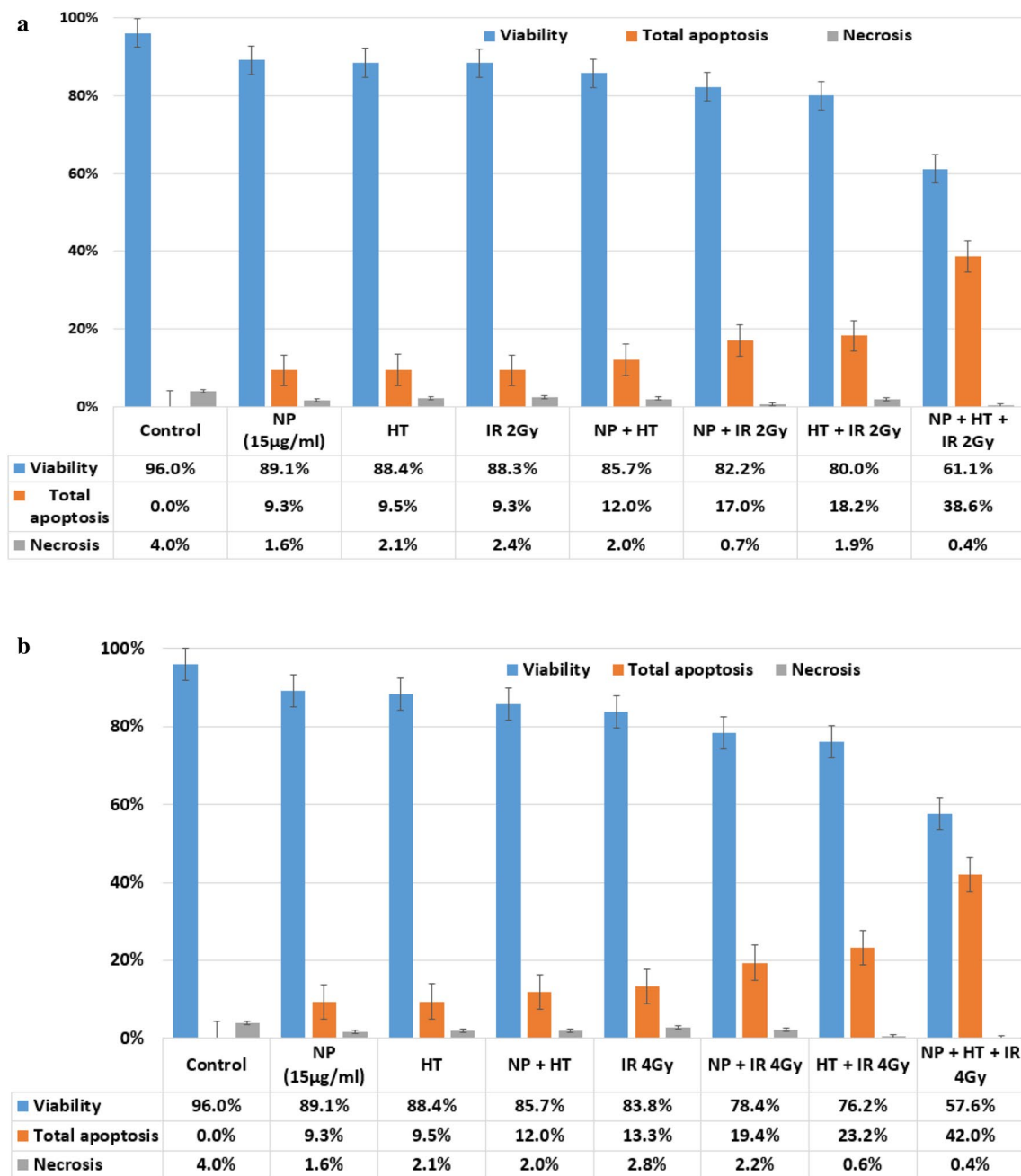


Fig. 11 Proportion of apoptotic and necrotic cell death for U87MG cells after receiving various treatment regimens; **a** 2 Gy and **b** 4 Gy (Error bars show means \pm SD). NP, HT and IR stand for nanoparticle, hyperthermia, and ionizing radiation, respectively

the effect of both 2 and 4 Gy of X-rays given alone. These findings are in agreement with other studies listed in Table 2.

Conclusions

In this study, we synthesized and characterized the Au@IONP nanocomplex and investigated its potential as adjuvant of hyperthermia and radiotherapy in glioma therapy.

Our results demonstrated that Au@IONPs potentiate the effect of hyperthermia and radiation suggesting that gold-coated iron oxide nanoparticles in combination with hyperthermia and radiation are a promising factor in treatment of therapy-resistant gliomas.

Table 1 Summary of the results obtained from flow cytometry analysis to determine if the various combination treatments have a synergic or additive effect

Group	Cell viability	Result	
NP (15 µg/mL)	89%	Synergic	Additive
[NP + HT] × IR (2 Gy)	Expected value: 85.7% × 88.3% = 75.7% Measured value: 61.1%	×	–
[NP + HT] × IR (4 Gy)	Expected value: 85.7% × 83.8% = 71.8% Measured value: 57.6%	×	–
[NP + IR (2 Gy)] × HT	Expected value: 82.2% × 88.4% = 72.7% Measured value: 61.1%	×	–
[NP + IR (4 Gy)] × HT	Expected value: 78.4% × 88.4% = 69.3% Measured value: 57.6%	×	–
[HT + IR (2 Gy)] × NP	Expected value: 80% × 89.1% = 71.3% Measured value: 61.1%	×	–
[HT + IR (4 Gy)] × NP	Expected value: 76.2% × 89.1% = 67.9% Measured value: 57.6%	×	–
NP × IR × IR (2 Gy)	Expected value: 89.1% × 88.4% × 88.3% = 69.6% Measured value: 61.1%	×	–
NP × IR × IR (4 Gy)	Expected value: 89.1% × 88.4% × 83.8% = 66% Measured value: 57.6%	×	–

NP nanoparticle, IR ionizing radiation, HT hyperthermia

Table 2 Summary of results from the current and other in vitro studies on the combined effect of AuNPs, hyperthermia and ionizing radiation

References	Cancer cell line	NP type	IR	HT	Apoptosis ratio (%)			
					NP	NP+HT	NP+IR	NP+HT + IR
Gao et al. (2015)	HepG ₂ Liver	Silica-coated@ Au-nanoroads	Iodine 125 seeds-35 keV	–	~ 21	–	~ 33	–
Chen et al. (2012)	MCF-7 Breast	Fe ₃ O ₄ NPs	–	MFH; 230KHz; 28A	0	25	–	–
Zhang et al. (2016)	4T-1 Breast	Au nanocages	X-ray; 6MV; 4 Gy	Laser; 808 nm; 2.5W/cm ²	–	6.5	10.8	20.8
Li et al. (2015)	A375 Skin	Gold nanoroads	X-ray; 6MV; 4 Gy	Laser; 808 nm; 1W/cm ²	2.5	4	15	25
Current study	U87-MG Brain	Au@IONPs	X-ray; 6MV; 4 Gy	Water bath hyperthermia (43°, 1 h)	5.9	3.5	11.2	38.2

NP nanoparticle, IR ionizing radiation, HT hyperthermia, MFH magnetic field hyperthermia

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Compliance with ethical standards

Conflict of interest A. Neshasteriz declares that he has no conflict of interest. Z. Khosravi declares that she has no conflict of interest. H. Ghaznavi and A. Shakeri-Zadeh declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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