Original research

The effects of folic acid-functionalized gold nanoparticles together with different irradiations on the peripheral blood mononuclear cells from cancerous and normal persons

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Abstract

The aim of this study was to evaluate the cytotoxicity, uptake, and reactive oxygen species (ROS) generation of folic acid-functionalized gold nanoparticles together with microwave (MW), ultra violet (UV), infrared (IR), UV+IR, and without any irradiation (darkness) on the peripheral blood mononuclear cells of chronic myeloid leukemia (CML) patients and normal persons. First, 5 CML patients and 5 normal persons were enrolled, and their peripheral blood mononuclear cells were isolated by Ficoll. Then, folic acid-functionalized gold nanoparticles were synthesized, added to cancerous and normal cells, and incubated for one hour at 37 ºC. In another experiment, the cells were incubated with the nanoparticles for one hour at 37 ºC, and then exposed to MW, UV, IR, and UV+IR. Finally, different assays, e.g. MTT, LDH, Alamar blue, uptake, and ROS generation were done. It was found that the cytotoxicity, uptake, and ROS generation of folic acid-functionalized gold nanoparticles was higher in the cancerous than normal cells. This study showed that all type of irradiations increased cytotoxicity, uptake, and ROS generation. At same concentration of folic acid-functionalized gold nanoparticles, the order of effects was MW > UV + IR > UV > IR > Darkness. The best efficacy score was 2.64, for concentration of 50 µg/mL nanoparticles together with IR. Folic acid-functionalized gold nanoparticles had higher toxicity on the cancerous than normal cells, and different irradiations (MW, UV, IR, UV+IR) raised their toxicity on the both cancerous and normal cells.

Keywords: Cytotoxicity; Folic acid-functionalized gold nanoparticles; Irradiation; Chronic myeloid leukemia

Introduction

Gold nanoparticles are widely used for different applications in biology and medicine, e.g. detection of cancer cells, drug delivery, imaging, etc [1-3]. Their wide range of applications is due to precise control on the size, shape, and surface chemistry [4]. Interestingly, some unique physical and chemical properties are observed when gold nanoparticles are treated with irradiation. Electromagnetic wave, at a specific wavelength, can stimulate electron conduction band of gold atoms, and generates a collective coherent oscillation, which leads to high absorption and scattering properties [5-7]. The adsorbed energy is converted into localized heat, which can be used for treatment of local cancer by hyperthermia. Based on previous studies, gold nanoparticles can increase the temperature of medium when exposed to near infrared (NIR) light [8, 9]. Moreover, gold nanoparticles can adsorb the energy of microwave (MW) irradiation, and the energy increases temperature [10, 11]. Other nanoparticles such as iron oxide nanoparticles and carbon nanotubes can generate heat after exposure to MW, too [12-14]. Although the increase of heat is the important behavior of gold nanoparticles after exposure with electromagnetic wave, the generation of active molecules such as hydroxyl radical (OH^{-}), superoxide anion (O_2^{-}), and ions is another behavior [15, 16]. Based on our previous work, gold nanoparticles generate reactive oxygen species (ROS) when exposed to ultra violet (UV) and infrared (IR) [17]. Importantly, ROSs are extremely active molecules and can kill different cancerous cells [18]. Note, an inverse ratio has been seen between ROS generation and the size of nanoparticles, which indicates importance of catalytic function of nanoparticle surface [19]. The killing of cancer cells may be done by the increase of temperature or by generation of ROS, but it must be mentioned that normal cells can be affected by both of them. To solve the problem, the killing agents must target the cancerous cells. Some molecules are over expressed on the cancerous cells which are used for targeted-drug delivery [20], e.g. folate receptor [21-25]. Note, with the method, the side effects of nanoparticles will be minimized. In our previous

study, we synthesized folic acid-functionalized gold nanoparticles and folic acid-functionalized silver nanoparticles, and their cytotoxicity was evaluated on the acute myeloid leukemia (AML) cells with and without MW [26]. In this study, we worked on the cytotoxicity, uptake, and ROS generation of folic acid-functionalized gold nanoparticles together with and without different irradiations (including MW, UV, IR, and UV+IR) on the blood samples of chronic myeloid leukemia (CML) and normal persons.

Methods

Materials

$HAuCl_4$ and folic acid were purchased from Merck, Germany. 3-(4,5-Dimethylthiazol-2)-2,5diphenyltetrazolium bromide (MTT), Hank's buffered salt solution (HBSS), 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate acetyl ester (CM-H2DCFDA), and RPMI1640 were provided from Sigma-Aldrich Chemical Co, (St Louis, MO, USA). Isopropanol (70% v/v) and NaOH were sourced from Zyst Fannavar Shargh Company (ZFS Co.), Yazd, Iran. Alamar blue reagent, LDH kit, and CellTiter-Glo Luminescent reagent were purchased from Invitrogen, UK.

Preparation of samples and obtain peripheral blood mononuclear cells

Five patients with CML and five normal persons which signed an informed consent form were enrolled in this study. The age of normal and CML persons was 20-50 years old, and all were male, with no smoking habits. It must be noted that the matching between patients and controls was considered. The classification of patients was done by Dr. Hekmatimoghaddam, as a clinical

pathologist, by means of routine histopathology methods including Giemsa staining and immune histochemistry. All of patients had CML with blastic phase, and did not receive any drug or treatment. In this study, 5 mL of venous blood was obtained, placed in heparinized propylene plastic tubes, and continuously shacked for 5 minutes. Then, peripheral blood mononuclear cells were isolated by Ficoll method [26]. Finally, the concentration of cells for each sample was adjusted to 1000 cells/mL by RPMI1640.

Synthesis and characterization of folic acid-functionalized gold nanoparticles

Firstly, 1 gram of folic acid was dissolved in one mL of deionized water, and then 100 microliter of 1 M NaOH was added to complete dissolution. Then, 5 mL of 1 mM $HAuCl_4$ and 1 mL of 1 g/mL folic acid were mixed, and incubated at 50 ºC for 10 hours (Figure 1a). The excess of $HAuCl_4$ and folic acid was removed by centrifugation at 10000 rpm for 15 minutes, washed with deionized water, dried at 37 ºC, and then the concentration of 200 µg/mL was prepared in deionized water by means of ball-miller (ZFS Co. Yazd, Iran). The structure and shape of folic acid-functionalized gold nanoparticles were characterized by scanning electron microscopy (SEM) (Hitachi S-2400, Japan) at 15 KV, and the size distribution of them was analyzed by dynamic light scattering (DLS) (ZFS Co., Yazd, Iran). On the other hand, their chemical composition was evaluated by Fourier transform infrared (FTIR) (ZFS Co., Yazd, Iran) at 500-4000 cm^{-1}. In this study, the spectrum of gold nanoparticles (100 µg/mL), folic acid (100 µg/mL), and folic acid-functionalized gold nanoparticles (200 µg/mL) were achieved.

The treatment of peripheral blood mononuclear cells with nanoparticles and different irradiations

First, 100 µL of folic acid-functionalized gold nanoparticles was separately added to 100 µL of suspension of peripheral blood mononuclear cells of each sample. Then, cells were incubated for one hour at 37 ºC. In another experiment, folic acid-functionalized gold nanoparticles (100 µL) and cell suspension (100 µL) of each sample were incubated for one hour at 37 ºC, and then separately exposed to darkness, UV, IR, (UV+IR), MW irradiation. The exposure time for UV irradiation (200-300 nm, 8 watt, Ultra-LumInc, USA), IR irradiation (800-1400 nm, 8 watt, Tungsram-Schreder, Hungary), (UV+IR), and darkness was 10 minutes. But the exposure time for MW irradiation (2450 MHz, 1225 W, MC-8041PVR, South Korea) was 20 seconds. The temperature of wells was measured during experiments by an electrical thermometer (ZFS Co.), and the highest temperature was recorded. It must be noted, serial concentrations (400, 200, 100, 50, and 25 µg/mL) of folic acid-functionalized gold nanoparticles were used for MTT assay, but for other assays (LDH assay, Alamar blue assay, ATP assay, uptake assay, ROS assay) only the concentration of 100 µg/mL were applied.

Peripheral blood mononuclear cells which were not exposed to folic acid-functionalized gold nanoparticles and irradiation were considered as negative control. On the other hand, because colorimetric and fluorescence test can be impaired by the nanoparticles, the solution containing different concentrations of folic acid-functionalized gold nanoparticles in the absence of cells was considered for all assays. Moreover, in order to correctly evaluate the interaction and the possible therapeutic value of irradiation, the cells were challenged also with the different type of irradiation in absence of any nanoparticle treatment.

MTT assay

After incubation, peripheral blood mononuclear cells were washed with HBSS, in order to remove any folic acid-functionalized gold nanoparticles. Then, 25 µL of 5 mg/mL MTT and 100 µL of RPMI1640 were added, and incubated for 3 hours at 37 ºC. Then, 100 µL of 70% v/v isopropanol was added, and optical density of each well was read at 492 nm by an ELISA reader (Novin Gostar Co., Iran). Finally, the cell death percentage and the efficacy score was measured for each group, according to Formula 1 and Formula 2, respectively.

Formula 1.

Cell death (%)= (Optical density$_{control}$-Optical density$_{test}$)×100/Optical density$_{control}$

Formula 2.

Efficacy score = the mean cell death of cancerous cells/ the mean cell death of normal cells

LDH assay

After incubation, peripheral blood mononuclear cells were centrifuged at 1500 g for 15 minutes, and supernatant of each sample was isolated, and the quantity of LDH enzyme in the supernatant was measured by a certified LDH kit. Briefly, 10 µL of the supernatant was added to reagent 1 (pyruvate in phosphate buffer) and reagent 2 (NADH in Good's buffer), and incubated for 5 minutes. Finally, the mean optical density of each test sample was read at 340 nm, and then divided to mean optical density of control sample (normalized to negative control).

Alamar blue assay

After incubation, peripheral blood mononuclear cells were rinsed with HBSS, and then 100 µL of RPMI1640 and 25 µL of the Alamar blue reagent were added and incubated 3 hours at 37 ºC.

Finally, the optical density of each sample (tests and control) was read at 590 nm using microplate reader (Novin Gostar, Iran), and then normalized to negative control same as LDH assay.

ATP assay

After incubation, peripheral blood mononuclear cells were washed with HBSS, and then 100 µL of CellTiter-Glo reagent and 100 µL of RPMI1640 were added and incubated for 10 minutes at 37 ºC. Finally, the light intensity of each sample was read by a luminometer (Turner Biosystems, model 9100-102, USA), and then normalized to negative control, same as LDH and Alamar blue assay.

ROS generation

First, 100 µL of the peripheral blood mononuclear cells from each blood samples was pretreated with 50 µL of CM-H2DCFDA at concentration of 10 µM, incubated at 37 °C for 1 hour, and treated with 100 µg/mL folic acid-functionalized gold nanoparticles together with and without irradiation. In the next step, peripheral blood mononuclear cells were washed three times with cold HBSS. Finally, the fluorescence intensity of each tube was read by Cytofluor series 4000 plate reader (PerSeptive Biosystems, Inc., Framingham, USA) at 485 nm excitation and 530 nm emission. All results were normalized to negative control, same as LDH, Alamar blue, and ATP assay.

Uptake assay

After incubation, peripheral blood mononuclear cells were washed 5 times with HBSS, and then 100 µL of 5 M HCl was added, and incubated for 24 hours at 37 ºC. Finally, the quantity of up taken folic acid-functionalized gold nanoparticles was quantified (µM) by atomic adsorption spectroscopy (AAS) (Biosystems, USA).

Statistical analysis

All assays were done on the five CML blood samples and five normal blood samples, and all assays were repeated three times for each sample. The results were shown as mean ± standard deviation (SD), and then parametric test (Student's t-test) was applied to evaluate the significant differences. In this study, SPSS software (V.16.0 for Windows; SPSS Inc., Chicago, IL) was used, and $P<0.05$ value was considered as statically significant difference.

Results

Characterization of folic acid-functionalized gold nanoparticles

The SEM image and DLS graph of folic acid-functionalized gold nanoparticles are shown in Figure 1b and Figure 1c, respectively. It is shown that the structure and shape of folic acid-functionalized gold nanoparticles is approximately spherical, and the size distribution of them is near 30-50 nm. The FTIR spectrum of gold nanoparticles (I), folic acid (II), and folic acid-functionalized gold nanoparticles (III) is shown in Figure 1d. Gold nanoparticles have specific peaks at 600 cm^{-1}, 1100 cm^{-1}, 1300 cm^{-1}, 1600 cm^{-1}, and folic acid at 1605 cm^{-1}, 1635 cm^{-1}, 3400 cm^{-1}, and 3540 cm^{-1}. Overall, all of peaks are observed at the spectrum of folic acid-functionalized gold nanoparticles, which indicated the conjugation of folic acid and gold nanoparticles.

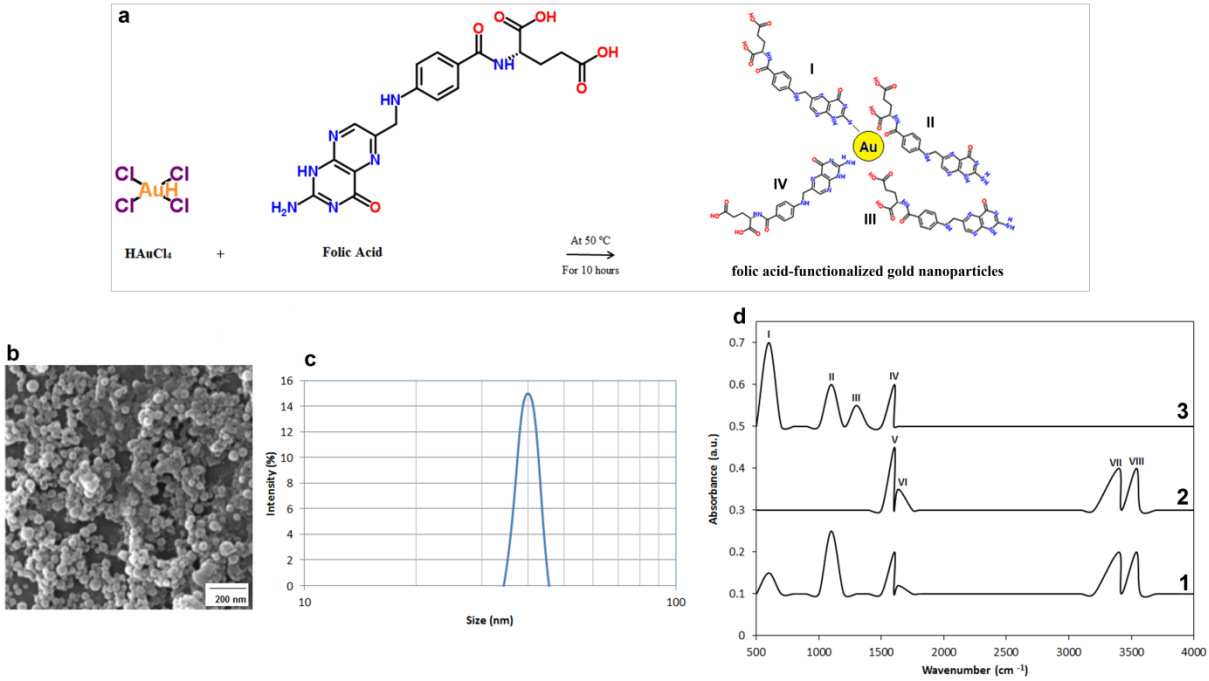

Figure 1. The schematic of reaction between folic acid and $HAuCl_4$ (a)**.** Gold can be conjugate by covalent attachment (I) and coordination band (II, III, IV). The SEM image (b) and DLS graph of folic acid-functionalized gold nanoparticles (c). The FTIR spectrum (d) of gold nanoparticles (1), folic acid (2), and folic acid-functionalized gold nanoparticles (3). Gold nanoparticles had specific peaks at 600 cm^{-1} (I), 1100 cm^{-1} (II), 1300 cm^{-1} (III), and 1600 cm^{-1} (IV). Folic acid had specific peaks at 1605 cm^{-1} (V), 1635 cm^{-1} (VI), 3400 cm^{-1} (VII), and 3540 cm^{-1} (VIII). Folic acid-functionalized gold nanoparticles had I-VIII peaks.

The result of MTT assay

Figure 2a, Figure 2b, Figure 2c, Figure 2d, and Figure 2e show the cell death of folic acid-functionalized gold nanoparticles together with MW, UV+IR, UV, IR, and no irradiation (at darkness), respectively, obtained by MTT assay. As shown at all part of Figure 2 (a-e), the

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cytotoxicity of folic acid-functionalized gold nanoparticles was dose-dependent, i.e. the higher concentration, the higher cell death. Mostly, significant differences were seen between concentration of 12 vs. 25 µg/mL, 25 vs.50 µg/mL, 50 vs. 100 µg/mL, and 100 vs. 200 µg/mL. As important finding which was seen at all part of Figure 2, the cytotoxicity of folic acid-functionalized gold nanoparticles on the cancerous cells was higher than normal cells ($P<0.05$). It must be noted that this pattern was seen at all concentration. In case of MTT results, to compare the effect of different irradiations, we designed Figure 3a-e which shows the effect of various irradiations together with folic acid-functionalized gold nanoparticles at concentration of 12, 25, 50, 100, and 200 µg/mL, respectively. As demonstrated, at the same concentration of folic acid-functionalized gold nanoparticles, the cancerous cells which exposed to MW irradiation had the highest cell death. Also, at the same concentration of folic acid-functionalized gold nanoparticles, the normal cells which not exposed to any irradiation had the least cell death.

Generally, the order of cell death at same concentration for both cancerous and normal peripheral blood mononuclear cells was: MW>UV+IR>UV>IR>darkness. Mostly, significant differences were seen between MW vs. UV+IR, UV+IR vs. UV, UV vs. IR, and IR vs. darkness ($P<0.05$). We also observed that the cytotoxicity of folic acid-functionalized gold nanoparticles on the cancerous peripheral blood mononuclear cells was more than normal peripheral blood mononuclear cells ($P<0.05$).

The results of efficacy score

Table 1 shows the efficacy score of different concentrations of folic acid-functionalized gold nanoparticles together with and without irradiations. It showed that the best efficacy score was 2.6, for combination of folic acid-functionalized gold nanoparticles at concentration of 50 µg/mL and IR

irradiation. Interestingly, folic acid-functionalized gold nanoparticles at concentration of 25, 50, and 100 µg/mL without any irradiation were in second level (with efficacy score of 2.5). On the other hand, the worst efficacy score was 1.1, after exposure of 12 µg/mL folic acid-functionalized gold nanoparticles and MW irradiation.

Table 1. The efficacy score of different concentrations of folic acid-functionalized gold nanoparticles together with various irradiations and without irradiation, obtained by MTT assay.

Concentration of the nanoparticles (µg/mL)	MW for 20 sec.	UV+IR for 10 min.	UV for 10 min.	IR for 10 min.	at darkness
12	1.1	1.5	1.9	2.3	2.2
25	1.2	1.4	1.8	2.2	2.5
50	1.1	1.2	1.5	2.6	2.5
100	1.2	1.2	1.5	2.1	2.5
200	1.2	1.2	1.4	1.6	2.3

The result of LDH, Alamar blue, and ATP assays

The results of other cytotoxicity assays including LDH assay, Alamar blue assay, and ATP assay are shown in Figure 4a, Figure 4b, and Figure 4c, respectively. In case of all assays, there were significant differences between the values of cancerous and normal cells ($P<0.05$). They demonstrated that different irradiations differently affected normal or cancerous cells. Generally, the order of cell cytotoxicity for both cancerous and normal cells was: MW>UV+IR>UV>IR>darkness, i.e. the highest LDH release, the least metabolic function, and the

least ATP level were seen for folic acid-functionalized gold nanoparticles together with MW irradiation. In contrast, the least LDH release, the highest metabolic function and ATP level were observed for folic acid-functionalized gold nanoparticles without any irradiation.

The results of ROS generation and uptake

The ROS generation and uptake of folic acid-functionalized gold nanoparticles together with different irradiations are shown in Figure 4d and Figure 4e, respectively. Interestingly, with and without irradiations, the cancerous cells had more uptake and ROS generation than normal cells ($P<0.05$). The maximum ROS generation and uptake was seen for cancerous cells when exposed to folic acid-functionalized gold nanoparticles and MW irradiation. The minimum ROS generation and uptake was observed for normal cells when exposed to folic acid-functionalized gold nanoparticles at darkness. The order of ROS generation and uptake for both cancerous and normal cells was: MW>UV+IR>UV>IR>darkness,

The results of temperature measurement

Figure 5 shows the final temperature of wells which were contained the cells and folic acid-functionalized gold nanoparticles at serial concentrations. As mentioned, different irradiations were applied. It experiment showed that MW led to increase of temperature to 70 ºC, and the concentration of nanoparticle could not remarkably affect it. Both UV+IR and IR had same pattern of temperature. As seen, increase of concentration led to increase of temperature. On the other hand, both UV and darkness had no effect on the temperature, and same pattern was seen for both. Note, the starting temperature for all wells was 37 ºC, and the surrounding ambient temperature during exposure was 25 ºC.

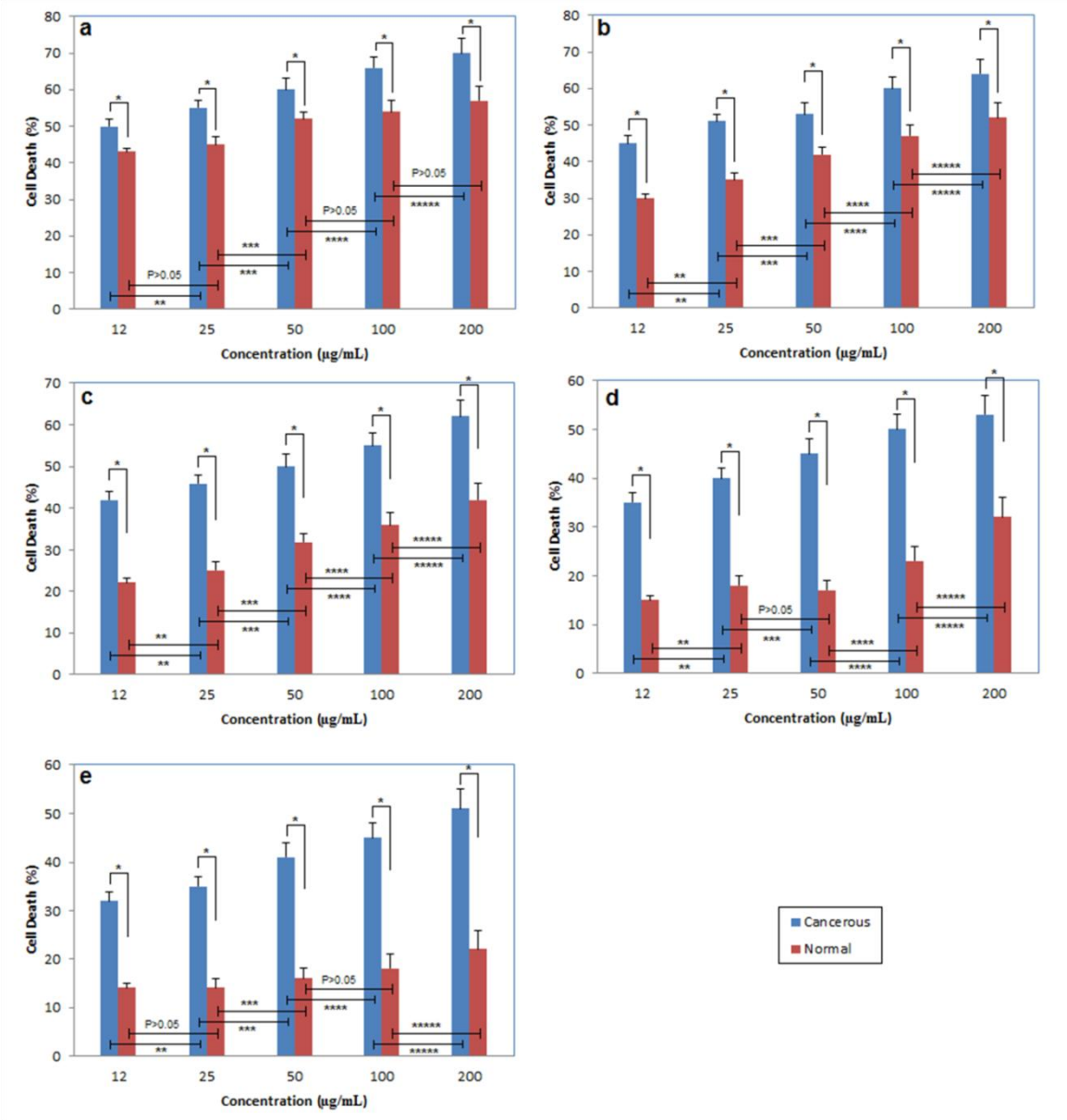

Figure 2. The effect of serial concentrations of folic acid-functionalized gold nanoparticles together with MW (a), UV+IR (b), UV (c), IR (d), and without irradiation (darkness) (e) on the CML and normal peripheral blood mononuclear cells. The evaluation of cytotoxicity was done by MTT assay. n= 15, * P<0.05 compared with cancerous and normal samples, ** P<0.05 compared with concentration of 12 µg/mL and 25 µg/mL, *** P<0.05 compared with 25 µg/mL and 50 µg/mL,

**** P<0.05 compared with 50 µg/mL and 100 µg/mL, ***** P<0.05 compared with 100 µg/mL and 200 µg/mL.

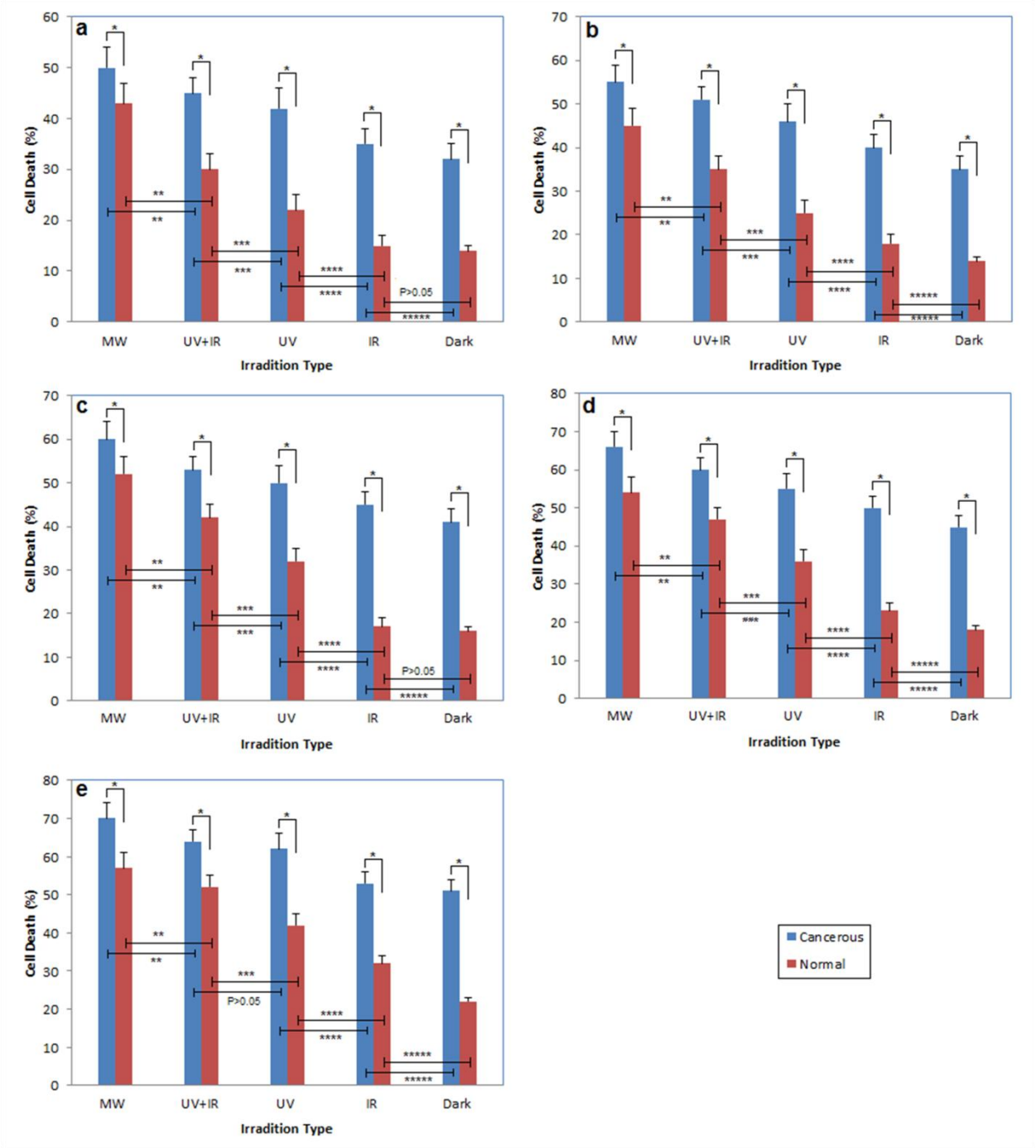

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Figure 3. The effect of different irradiations together with folic acid-functionalized gold nanoparticles at concentration of 12 µg/mL (a), 25 µg/mL (b), 50 µg/mL (c), 100 µg/mL (d), and 200 µg/mL (e) on the CML and normal peripheral blood mononuclear cells. The evaluation of cytotoxicity was done by MTT assay. n= 15, * $P<0.05$ compared with cancerous and normal samples, ** $P<0.05$ compared with MW and UV+IR, *** $P<0.05$ compared with UV+IR and UV, **** $P<0.05$ compared with UV and IR, ***** $P<0.05$ compared with IR and darkness.

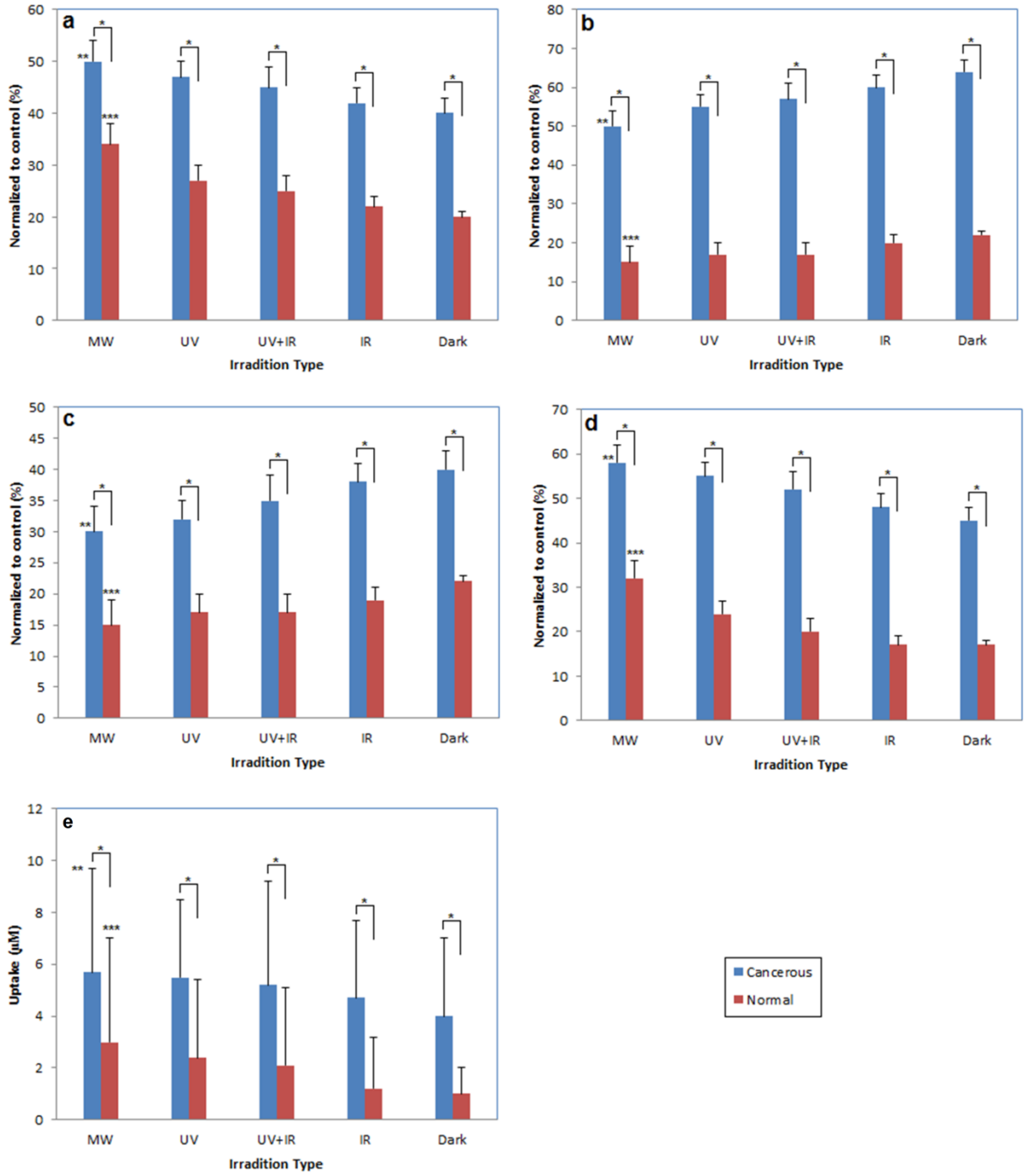

Figure 4. The effect of different irradiations together with folic acid-functionalized gold nanoparticles obtained by LDH assay (a), Alamar blue assay (b), ATP assay (c), ROS generation (d), and uptake assay (e). n= 15, * P<0.05 compared with cancerous and normal, ** P<0.05

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compared with darkness for cancerous samples, *** P<0.05 compared with darkness for normal samples.

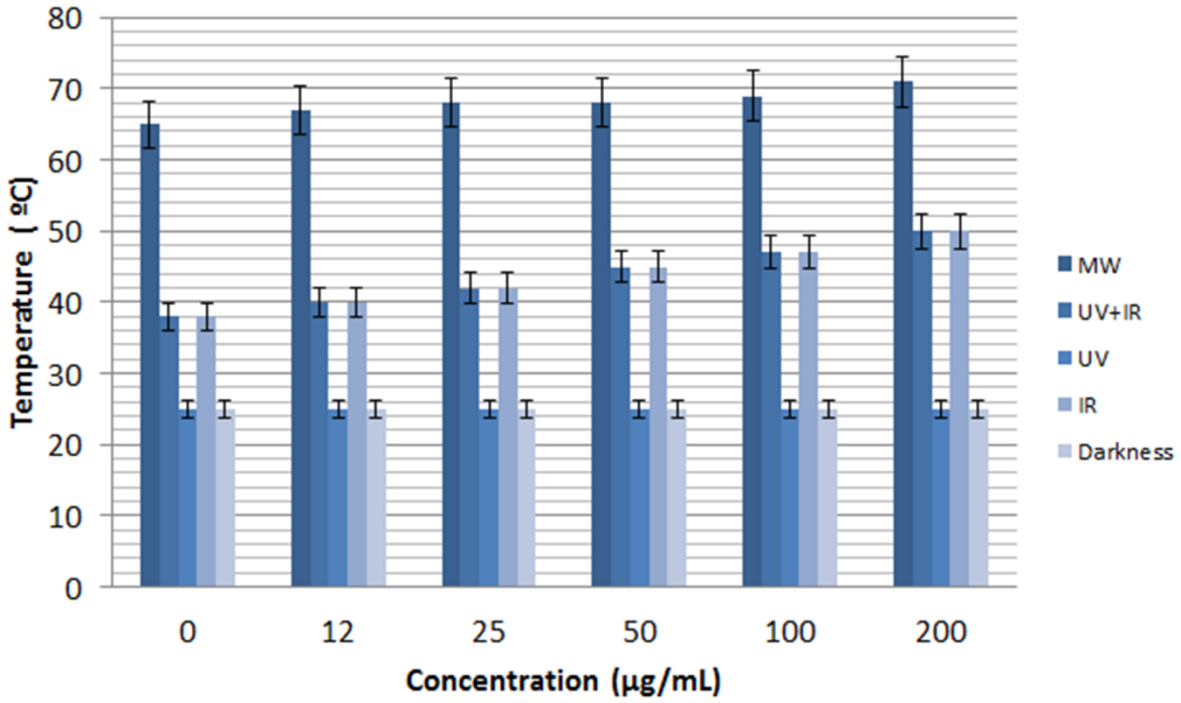

Figure 5. The final temperature after incubation of serial concentrations of folic acid-functionalized gold nanoparticles together with MW, UV+IR, UV, IR, and without any irradiation.

Discussion

The aim of this study was to investigate the cytotoxicity, ROS generation, and uptake of folic acid-functionalized gold nanoparticles together with and without various irradiations on the cancerous and normal cells. In this study, gold nanoparticles were selected, because the nanoparticles have a well-defined chemistry, and can be easily conjugated with different molecules [4-7]. Moreover, based on previous studies, gold nanoparticles have been used for detection and treatment of various cancers [1, 2]. Unfortunately, the nanoparticles can damage normal cells, same as cancerous cells,

and the problem is very important [3]. Regarding the issue, we conjugated folic acid on the gold nanoparticles, which named folic acid-functionalized gold nanoparticles. The aim of the use of folic acid on the gold nanoparticles was to target the cancerous peripheral blood mononuclear cells [27]. It is fact that folic acid receptor is over expressed on the various cancer cells, e.g. CML [21-23]. In this study, to synthesize folic acid-functionalized gold nanoparticles, the method of Li et al was used [28], because it is simple, cost-effective, and high output. Li et al declared that -NH2 group of folic acid can be conjugated with gold nanoparticles. Also, gold atom can interact with carboxyl and amine group of folic acid by coordination or chelation. Although we used Li et al method, other conjugation procedures could be applied, such as the use of cross-linker or spacer [24, 25]. Because the size of nanoparticle is an important factor for toxicological study [29], we propose that the effect of size must be studied in future studies.

Our hypothesis was that various irradiations can change cytotoxic effects, ROS generation, and the uptake of folic acid-functionalized gold nanoparticles. Theoretically, gold nanoparticles can adsorb energy of electromagnetic wave, and can be exited [5]. For example, the MW irradiation can directly vibrate water molecules and gold nanoparticles [10, 30], and the vibration increases ROS, kinetic energy, and the medium temperature. We suggest that both direct and indirect effects of MW irradiation lead to more cell cytotoxicity. On the other hand, gold nanoparticles can adsorb the energy of UV light, and generate free radical or gold ion to damage cells [15, 16]. Also, the IR light can vibrate gold atoms, and increases temperature of medium and generate ROS [8]. Generally, MW, UV, and IR irradiation increase the cytotoxic effects of gold nanoparticles by increase of temperature and/or generation of ROS.

This study had three distinct findings. The first was that the cytotoxicity of folic acid-functionalized gold nanoparticles on the cancerous cells was more than normal cells. The reason of the

phenomenon may be due to folic acid moieties which lead to targeted uptake of folic acid-functionalized gold nanoparticles. Interestingly, the uptake and ROS generation of folic acid-functionalized gold nanoparticles was in consistent with cytotoxicity results, i.e. the uptake and ROS generation of folic acid-functionalized gold nanoparticles in the cancerous cells was more than normal cells. It was shown the cytotoxic effects of folic acid-functionalized gold nanoparticles on the both normal and cancerous cells, and other uptake mechanisms might be included [29].

The second finding was that various irradiations led to different cytotoxic effects. At same concentration of folic acid-functionalized gold nanoparticles, the order of cytotoxicity was: MW > UV + IR > UV > IR > darkness. As seen, the cancerous cells which exposed to MW irradiation had the highest cell cytotoxicity and the normal cells which not exposed to any irradiation had the least cell cytotoxicity. We explain that MW, IR, and UV+IR lead to jump medium temperature, and this damage both cancerous and normal cells. It was shown that the combination of UV and IR light was more effective than UV or IR alone. The authors propose that UV light can generate free radical and gold ions, and IR light can generate heat. All of them can damage cells, and lead to cell death. It should be noted that the order (MW > UV + IR > UV > IR) can be changed easily, if different sources of irradiation with different powers and exposure times are used.

Third finding was that the best efficacy score (=2.64) was seen for the combination of folic acid-functionalized gold nanoparticles at concentration of 50 µg/mL and IR irradiation. Also, folic acid-functionalized gold nanoparticles at concentration of 25, 50, and 100 µg/mL without any irradiation had high efficacy score (near 2.5). We find that although irradiation can increase potential of folic acid-functionalized gold nanoparticles to kill cancerous cells, but it leads to decrease of efficacy score, and it is not good.

There is no same study in the literature, and the some related articles will be discussed in this paragraph. Hekmatimoghaddam et al worked on the rate of cell cytotoxicity induced by folic acid-functionalized gold nanoparticles and folic acid-functionalized silver nanoparticles with and without 2450 MHz MW irradiation on the AML cancerous cells. They found that MW irradiation led to increase of cytotoxicity of folic acid-functionalized gold nanoparticles and folic acid-functionalized silver nanoparticles. They declared that the efficacy score of folic acid-functionalized gold nanoparticles without irradiation was better than folic acid-functionalized gold nanoparticles together with irradiation [26]. Cardinal et al showed gold nanoparticles together with MW at frequency of 13.56 MHz could kill HepG2 liver cancerous cells [31]. Since the wave absorption property and dielectric constant of nanoparticles are important factors to adsorb MW energy, other nanoparticles have been used, too. In case of UV and IR irradiation together with nanoparticles, Jebali et al showed that the combination of UV or IR together with metal and metal oxide nanoparticles led to higher toxicity [17].

Regarding the clinical application of this study, the limited depth of penetration of UV and IR is a major obstacle, and 2450 MHz microwave is not approved for medical use. On the other hand, these irradiations lead to more toxicity of folic acid-functionalized gold nanoparticles. So, the nanoparticles should not apply together with any irradiation. We suggest that folic acid-functionalized gold nanoparticles must be used on the animal model of CML in the future.

Taken together, the folic acid-functionalized gold nanoparticles had higher toxicity on the cancerous cells than normal cells, and irradiation could increase their toxicity. The results of the study can be used for treatment of CML in future, but we have a long way to reach to it. The in vivo studies are important section to find more.

Conclusions

We found that folic acid-functionalized gold nanoparticles had higher toxicity on the cancerous mononuclear cells than normal mononuclear cells. Different irradiations (MW, UV, IR, UV+IR) not only increased the toxicity of the nanoparticles on the cancerous cells, but also on the normal cells. The results must be more investigated at future in vitro and in vivo studies.

Acknowledgments

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Conflict of interest

No conflict of interest is declared.

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