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RESEARCH ARTICLE

Investigation of the dose enhancement effect of spherical bismuth oxide nanoparticles in external beam radiotherapy

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ABSTRACT

Objective(s): External radiotherapy is the most common method of radiotherapy which the most important problem associated with is that there is no difference between healthy and tumor tissues in dose absorption. One way to differentiate the dose sensitivity is to use metal-based nanoparticles. Bismuth oxide nanoparticles are good candidates for cancer radiotherapy. In this study, the dose enhancement effect of the synthesized spherical Bi_2O_3 NPs was investigated in 6 MV external radiotherapy.

Methods: Bi_2O_3 were synthesized and GENIPIN gel dosimeter was produced and divided into two equal portions, one part to fill vials containing pure gel and the other part to be added to a specified amount of nanoparticles to give a concentration of 0.1 mM. Then, the irradiation of the pure gel and gel vials containing the NPs was performed one day after manufacture by a 6 MV external radiotherapy device. Gel readout was performed using spectrophotometer and absorption-dose curves were achieved.

Results: Results show that spherical Bi_2O_3 NPs cause a decrease in GENIPIN absorbance range compared to gel without NPs. The slope of the absorbance-dose curve in presence of NPs is-0.038 which is more than this slop in the pure gel (-0.029) which indicates a DEF of 1.31 in the usage of these NPs in the tumor.

Conclusions: We can conclude that by applying these spherical Bi_2O_3 NPs, dose absorption of the tumor will increase up to 31% which means the efficacy of radiotherapy can be maintained by lower applied dose to the tumor and healthy cells.

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INTRODUCTION

Radiation therapy is among the most common modalities in the treatment of cancer that aims to transfer the highest dose to the tumor and the lowest dose to the healthy tissues and vital organs. Today, more than half of cancer patients benefit from radiotherapy for treatment [1, 2] that could be performed through various techniques. External beam radiotherapy is the most common type of radiotherapy which the source of radiation is outside the patient's body. In this type of radiotherapy high-energy ionizing radiation is used. One of the fundamental problems in radiotherapy is that there is no discrepancy in the absorption of radiation in healthy and tumor cells (discrepancy in the radiosensitivity of healthy and tumor cells) so

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damage to healthy tissues is the limiting factor of the dose delivery. One of the effective treatments is to increase the sensitivity of the tumor cells to radiation. Metal-based nanoparticles are one of the radiation sensitizers of therapeutic agents that increase the radiosensitivity of tumor cells and the efficacy of radiotherapy [3].

Water is the main component of the cell and the primary target of ionizing radiation. X-ray absorption can ionize water molecules and create free radicals. These free radicals cause DNA damage in cancer cells. In addition, the reaction of free radicals with membrane structures can also cause structural damage and thus stimulate cell apoptosis [4, 5]. The surface of the nanoparticles causes the formation of hydroxyl free radicals, which is the primary target of radiation therapy [6]. Moreover, metal nanoparticles have high photoelectric cross section due to their high atomic number, according to the (Z^3/E^3), which E is the beam energy and Z is the atomic number of the target material. Auger electrons produced in the photoelectric phenomenon and in some cases by inelastic collisions (Compton) due to their short range (usually 10 nm) cause high ionization in the region [7]. Bismuth is a substance with a high atomic number (Z=83), thus increases the dose and improves the efficacy of radiotherapy [8]. Bismuth compounds have long been used in medicine to treat skin ulcers, Helicobacter pylori infection [9], contrast agents in CT scans, and the removal of drug-resistant bacteria [10]. Many bismuth compounds are biocompatible [11], low cost [12, 13] and less toxic than nanoparticles such as gold [14] and can be produced in various shapes and sizes [15]. Bismuth oxide nanoparticles have greater cell penetration power and fewer side effects than other conventional radiation sensors [16, 17]. Moreover, nanoparticle size is one of the most important factors in increasing absorption dose. As the diameter of the nanoparticles increases, the rate of production of secondary electrons also increases. These secondary electrons are partially absorbed by the nanoparticles. The absorption increases with increase in nanoparticle diameter. On the other hand, the use of small nanoparticles smaller than 10 nm is less likely to be exposed to radiation [18]. The size of the nanoparticles in addition to their effect on dose enhancement, also influences their penetration into the target tissue. The small size of the nanoparticles increases their excretion from the body's immune system and their large size prevents them from entering the cell wall [19]. This study aims at investigating the dose enhancement effect of 18-25 nm synthesized bismuth oxide nanoparticles in 6 MV energy using GENIPIN gel dosimeter.

One of the methods for measuring the dose changes in the presence of nanoparticles is the use of nanoparticles in water equivalent gel dosimeters [20]. GENPIN gel is a non-toxic dosimeter and according to studies, they are very similar to water and body tissue [21, 22]. GENIPIN gel undergoes radiochromic changes in response to ionizing radiation that can be read and evaluated by optical CT and spectrophotometer. Recently, few studies have been performed on the dose enhancement effect of bismuth oxide nanoparticles [7, 23]. Moreover, the size and shape of the nanoparticles and the environment they are studied in (gel dosimeters) have a different effect on this response. We investigated dose enhancement effect (DEF) of synthesized bismuth oxide nanoparticles for the first time with GENIPIN gel dosimeter in external beam radiotherapy.

MATERIALS AND METHODS

Bi_2O_3 synthesis:

Bi_2O_3 were synthesized by hydrothermal method via precipitation route. Spherical bismuth nanoparticles were made using a novel method to produce this form of nanoparticles.

First, 1 g of bismuth nitrate salt (99.9 %, Sigma-Aldrich) was combined with 12.5 ml of nitric acid (50- 70% Merck) on a heater stirrer, then 33 ml of ammonium hydroxide (Sigma Aldrich) solution was added to the mixture at once and blended thoroughly for 10 minutes on the heater stirrer.

The solution was then poured into the Falcon tube and centrifuged five times. Three centrifugation steps were performed at 4000 rpm for 5 minutes, the forth run at 2000 rpm for 5 minutes and the fifth run at 3500 rpm for 2 minutes. The remaining precipitate was mixed with 100 ml of deionized water and placed at 100 °C for 5 and half an hour (Fig. 1).

The mixture was finally transferred to two 50 ml Falcon tubes and centrifuged at 4000 rpm for 5 minutes. The residual precipitate of the nanoparticles was mixed with a little deionized water and poured into a small beaker. For complete drying of the nanoparticles, the compound was heated at 200 °C for 2 h. A small amount of nanoparticles went through FESEM and TEM tests.

Fig.1. Nanoparticles precipitate was mixed with 100 ml of deionized water and placed at 100 ° C for 5 and a half hours

GENIPIN gel dosimeter preparation

GENIPIN gel was synthesized using the optimal formula of Davis et al. [24] By combining 4% gelatin (Porcine skin, Sigma-Aldrich), 50 μM GENIPIN (98% ,Sigma-Aldrich) and 100 mM sulfuric acid (98% , Merck) presented in Table 1.

The gelatin was first mixed with water and stirred continuously on a stirrer at 45 °C until the gelatin was completely dissolved in water and a clear solution was obtained. Then GENIPIN was added to the mixture and the solution temperature was raised to 70 °C. The mixture was stirred on the heater stirrer for 5 hours at this temperature until the gelatin was fully combined with GENIPIN as cross-linker. During this time, GENIPIN changes from pale blue to dark blue (Fig. 2). After this period, sulfuric acid was added and stirred for 10 minutes.

Table 1. GENIPIN gel dosimeter compounds

Weight percent	compounds
4٪	Gelatin
50 μM	GENIPIN
100 mM	Sulfuric Acid
93%	Ultra pure water

GENIPIN is then divided into two equal portions, one part to fill vials containing pure gel and the other part to be added to a specified amount of nanoparticles to give a concentration of 0.1 mM and kept at 4 °C for 1 day.

Irradiation

The gels containing the nanoparticles and the vials of the pure gel were irradiated at the conventional dose of 2 Gy per fraction in external beam radiotherapy. Irradiation was performed by 6 MV photon beam produced by compact machine (Fig. 3).

To increase the measurement accuracy of each gel vial, four samples were taken for irradiations. For each gel group, a non-irradiated gel vial was considered for zero dose measurement. Gel vials and distilled water were placed in the irradiation room 1h before irradiation to reduce the effect of temperature difference on the gels. The gels were placed in the water phantom. The irradiation

Fig. 2. GENIPIN color changing from pale blue to dark blue (left to right) during preparation

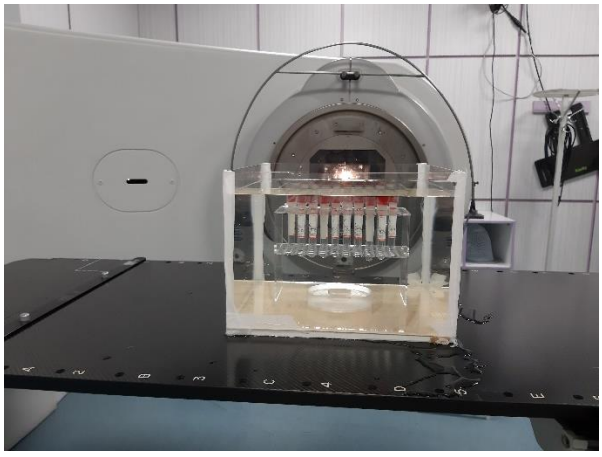

Fig. 3. Irradiation set-up using compact external beam radiotherapy machine

conditions include a 25×25 cm^2 field size and SSD = 100 cm. The monitor unit was set to deliver a dose of 2 Gy to the center of the gel vials.

Gel read out by spectrophotometer

The radiochromic changes in GENIPIN gel at its absorbance peak, which is 600 nm, were measured by a spectrophotometer. The gels were read using a spectrophotometer at wavelengths of 300 to 650 nm. The absorption curves of each vial were obtained separately and stored in Excel files. The gel absorption curves were plotted at zero and 2 Gy and according to the absorption peak of this gel, the absorption-dose curves of each vial were obtained.

Analysis

The slope ratio of absorption-dose curve in the presence of nanoparticles to the pure gel indicates the dose enhancement factor (DEF). DEF was extracted from the absorption-dose curves.

RESULTS AND DISCUSSION

The morphology, shape and size of the nanoparticles were investigated using TEM and FESEM.

The Fig.4 depicts FESEM and TEM of the synthesized bismuth oxide nanoparticles.

The dimension of bismuth oxide nanoparticles in FESEM images is about 21-36 nm and according to the image of TEM analysis it is about 18-25 nm. The reason for the larger particle size in FESEM images is the gold coating that is created on the nanoparticles during the sample preparation for FESEM analysis. In this type of nanoparticles, due to the high tendency to create nanowire structures, the larger dimensions lead to the creation of nanowire structures that are formed during the growth of energetic plates. These spherical nanoparticles are the primary nuclei of the nanoparticles.

Fig. 5 shows the absorption-wavelength curve of GENIPIN gel read by spectrophotometer. As shown in the curve, with increasing absorption dose, a decrease in optical absorption of GENIPIN is seen. The peak of optical absorbance occurred at 602 nm, which is consistent with previous studies [24] which indicates the correctness of the process of gel production.

Fig. 6 shows the absorption-wavelength curve of GENIPIN gel in the presence of 0.1 mM of spherical bismuth oxide nanoparticles. In the presence of nanoparticles, a further decrease in the absorption peak height with increasing dose was observed. Using nanoparticles in the gel did not alter the location of the absorption peak, which somehow indicates the non-solubility of the nanoparticle in the gel composition and its stability in the gel matrix structure.

The absorbance-dose curve containing the nanoparticles at 0.1 mM concentration compared to the pure gel is shown in Fig. 7.

As shown in the Fig. 7, the use of nanoparticles also showed a further decrease in the adsorption range of 2 Gy, indicating an increase in the absorption dose in the presence of the nanoparticles. The slope of the absorption-dose curve of pure gel is -0.029 and the slope of the curve in presence of spherical nanoparticles is -0.038.

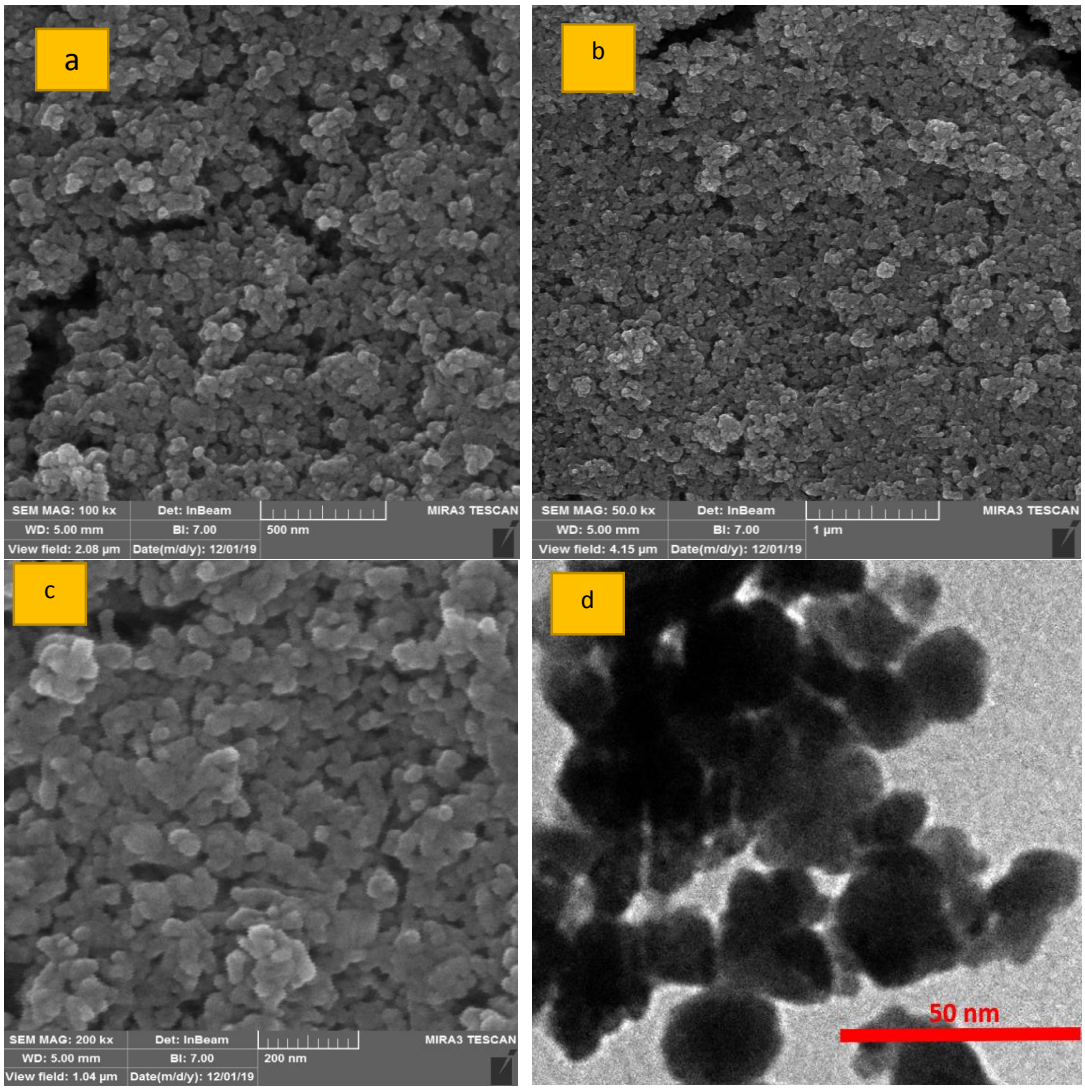

Fig. 4. a, b, c) FESEM of spherical Bi_2O_3 NPs and d) TEM of spherical Bi_2O_3 NPs

Fig. 8 shows mean DEF in the presence of spherical bismuth oxide nanoparticles with a concentration of 0.1 mM. As shown in this figure, DEF in the presence of nanoparticles is equal to 1.31 at 6 MV photon irradiation.

In recent years, some studies have been done on metal nanoparticles especially bismuth compounds. In 2016, Stewart et al. conducted a study on bismuth oxide nanoparticles as radiation sensitizers. Radiation-resistant cells of gliosarcoma 9L were exposed to 50 µg/mL of Bi_2O_3 nanoparticles prior to irradiation. DEF for 125 kV and 10 MV energies was 1.48 and 1.25 respectively. The results of this study show that bismuth oxide nanoparticles increase the radiosensitivity of gliosarcoma 9L cells in both the 125 kV and 10 MV energy. However, applying spherical bismuth oxide synthesized in this study increased DEF to a greater extent. Another study performed by Farahani et al. in 2019 investigated the dose enhancement effect of 0.2 mM gold nanoparticles in external radiotherapy by Co-60 in MAGAT gel dosimeter [25]. They found that DEF increased about 5.85% in the presence of gold NPSs in 1.25 MV energy. More over in another study conducted by them, the DEF enhancement of 0.2 mM gold and bismuth nanoparticles irradiated by Co-60 in nPAG gel dosimeter were less than 4% [26]. In this study, DEF was approximately

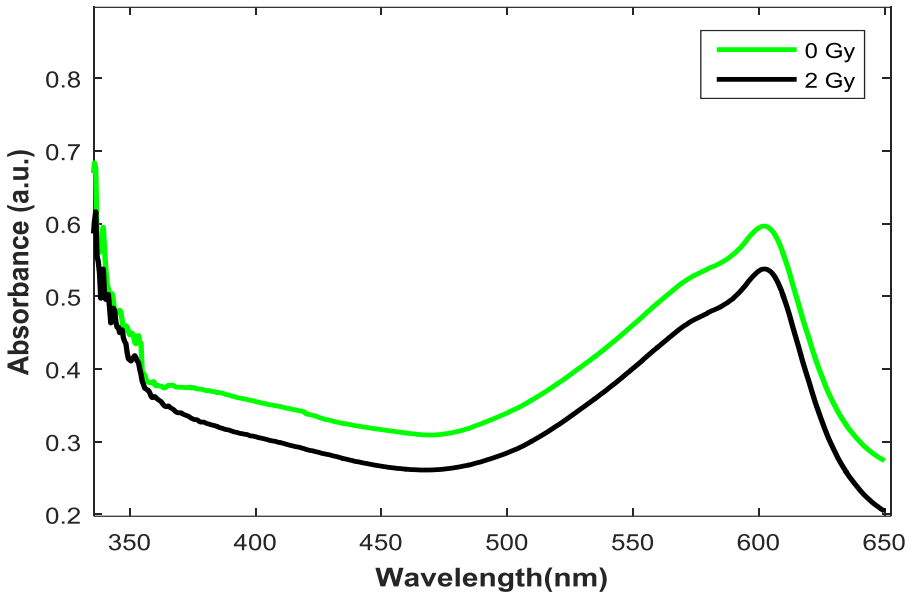

Fig. 5. Absorption-wavelength curve of GENIPIN gel read by spectrophotometer

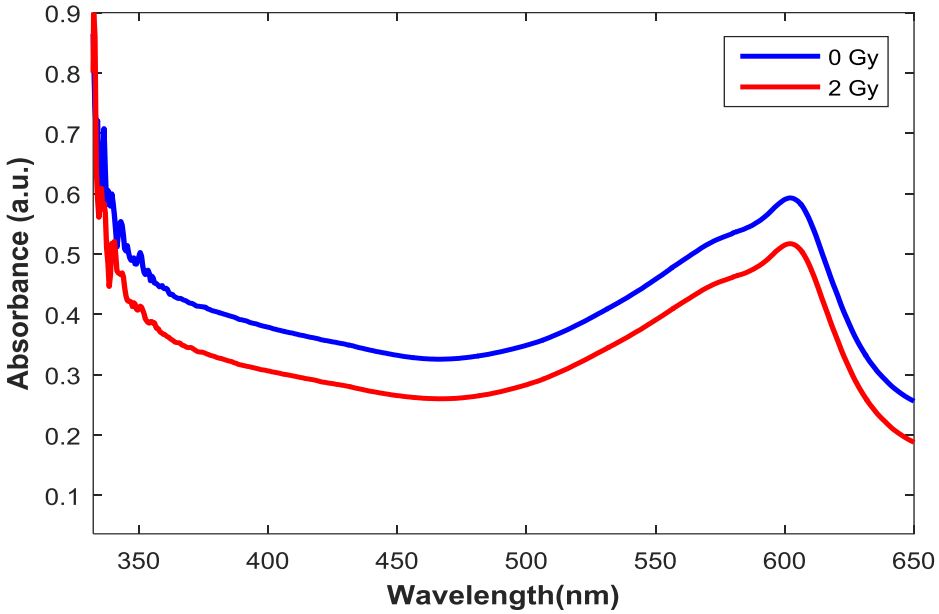

Fig. 6. Absorption-wavelength curve of GENIPIN gel in the presence of 0.1 mM of spherical bismuth oxide nanoparticles

27% higher than studies done by Farahani et al. This difference may be due to several reasons. First, bismuth oxide nanoparticles have an atomic number greater than pure gold and bismuth, so the likelihood of photoelectric phenomena in the use of these nanoparticles increases. In addition, the gel environment with which the nanoparticles blend is of great importance. As observed in studies performed by Farahani et al. DEF values were different in identical nanoparticles and similar energies using two different gel dosimeters. GENIPIN was used in this study which is very unlikely to react with nanoparticles. So an increase in DEF was observed compared to studies

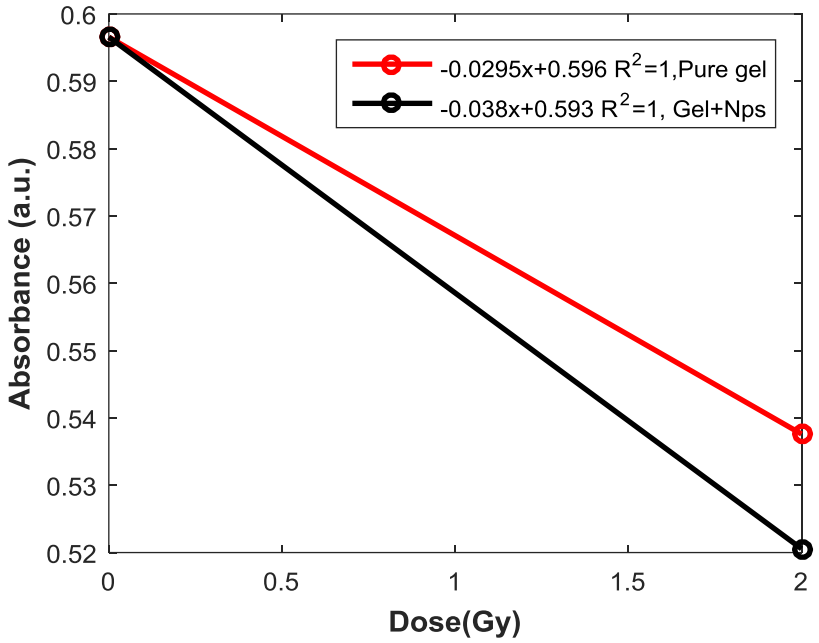

Fig. 7. Absorbance-dose curve containing the nanoparticles at 0.1 mM concentration compared to the pure gel

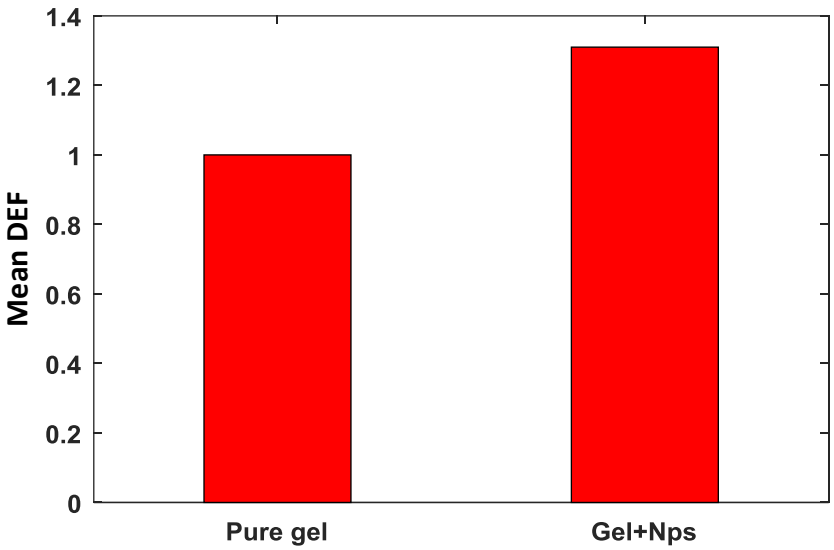

Fig. 8. Mean DEF in the presence of spherical bismuth oxide nanoparticles with a concentration of 0.1 mM

conducted by Farahani et al. It seems that choosing the appropriate gel dosimeter for investigating the DEF of nanoparticles is of great importance.

The results of this study by using GENIPIN gel dosimeter, shows that by using spherical bismuth oxide nanoparticles at a concentration of 0.1 mM at 6 MV photon beam, the dose to tumor can be reduced up to 31%.

CONCLUSION

Acceptable DEF in energy range of 6 MV with low concentrations of nanoparticles and as a result lower toxicity related to high concentration, was achieved in this study.

Finally, by reducing the transfer dose to the tumor and healthy tissue by up to 31% in the presence of the synthesized nanoparticles inside the

tumor, we can maintain the efficacy of external beam radiotherapy while decreasing the damage to normal tissues. Moreover, the risk of tumor recurrence will diminish significantly by maintaining the delivered dose in the presence of nanoparticles.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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