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RESEARCH PAPER

Au Nanoparticle Loaded with 6-Thioguanine Anticancer Drug as a New Strategy for Drug Delivery

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ABSTRACT

In this study we suggested a new strategy for drug delivery of 6-thioguanine (6-TG) as a cancer drug by loading of this thiolic drug at a surface of Au nanoparticles. For this goal, we synthesized Au nanoparticle (Au/NPs) by reduction of tetrachloroauric (III) acid solutions by sodium borohydride and characterized Au/NPs by X-ray powder diffraction (XRD), dynamic light scattering (DLS), Ultraviolet–visible spectroscopy (UV-Vis) and transmission electron microscopy (TEM) methods. Results showed good distribution of synthesized Au nanoparticle with diameter ~ 5 nm. In the second step, we loaded 6-thioguanine with strong Au-S bond by addition of this drug to Au nanoparticle suspension and characterized it by UV-Vis method. Furthermore, Au nanoparticle loaded with 6-thioguanine was utilized as a new system against breast cancer cell line (MCF7). IC_{50} for free 6-thioguanine, unloaded Au nanoparticle and 6-thioguanine loaded Au nanoparticle (6-TG/Au/NPs) were >15.0, 80.0 and 3.5 μg/ml, respectively. The results obtained revealed high cytotoxicity effect of 6-TG/Au/NPs on breast cancer cell line.

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INTRODUCTION

Drug delivery systems (DDSs) were suggested as high powerful tools for improving the efficacy of chemotherapeutics [1-4]. Application of DDSs can be reduced the side effect of anticancer drugs in chemotherapeutics [5, 6]. Materials in nanoscale such as nanocapsules, nanotubes, nanoparticles and nanosheets have been growing in DDSs as drug carriers [7-10]. Nanoscopic material help to DDSs for better release of the drugs with controlled fashion and more loading of anticancer drugs for chemotherapeutics [11, 12]. In addition, the nanoparticles with unique properties such as high surface area or magnetic properties can be useful for delivering high value of anticancer drugs and targeting delivery of anticancer drugs [13-15].

Au nanoparticles showed more attention in drug delivery systems due to low toxicity, the ability to formulate mixed monolayers, stable under most physiological conditions and high payload-to-carrier ratios [16]. Therefore, some published papers were reported application of Au nanoparticles for fabrication of novel DDSs [17-19]. On the other hand, strong Au-S bond is a good choice for loading of thiolic compounds at a surface of Au surface [20-22]. As example, Podsiadlo ***et al.,***

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reported application of Au nanoparticle for enhance the Anti-Leukemia Action of a 6-Mercaptopurine [23]. The suggested DDSs showed good activity on K-562 leukemia cells with high loading of 6-mercaptopurine as an anticancer drug.

6-Thioguanine (6-TG) is a purine derivative anticancer drug that used to treat acute myeloid leukemia, inflammatory bowel disease, acute lymphocytic leukemia, metastatic breast cancer and chronic myeloid leukemia[24]. Although, 6-TG was suggested as a useful anti-cancer drug it showed many side effects in the human body such as black, tarry stools, fever or chills, unusual bleeding or bruising, cough or hoarseness and pinpoint red spots on the skin. Therefore, it is very important for control reseals of this anticancer drug in chemotherapeutics.

According to the above points, in this study we fabricated a novel DDSs based on Au nanoparticles loaded with 6-TG anticancer drug. The proposed systems showed enhance the anti-cancer action on breast cancer cell line (MCF7).

MATERIALS AND METHODS

6-Thioguanine and tetrachloroauric(III) acid trihydrate were purchased from Fluka and Merck Company, respectively. Sodium borohydride, sodium hydroxide and sodium citrate decahydrate obtained from Sigma-Aldrich. For cell culture investigation RPMI 1640 medium was prepared from Gibco (BRL, Grand Island, NY, USA). The human breast cancer cells line (MCF-7) was prepared from national cell bank of Iran (Pasteur institute of Iran, Tehran) and cultured in RPMI 1640 medium supplemented with 10% heat inactivated FBS and 100 U/ml penicillin and 100 µg/ml streptomycin at 37°C in humidified incubator with 5% CO_2.

Zeiss EM900 Transmission Electron Microscope (TEM) and EQuniox 3000 diffractometer employed for investigating Au nanoparticles. A Cary-500 double-beam spectrophotometer was used for UV/Visible investigation.

Au nanoparticle and 6-TG loaded Au nanoparticle synthesis procedure

In the first step, tetrachloroauric(III) acid trihydrate (0.5 mL of 1 wt %) was diluted in 50 mL distillated water and stirred for 60 s. In continuous, 0.5 mL of 3.4 mM sodium citrate solution added to the previous solution and obtained solution stirred for 60 s. In the final step, Au nanoparticle was obtained by addition of 0.5 mL of 0.075 wt% sodium borohydride as reducing agent to the previous solution. The synthesized Au/NP was diluted with an additional 50 mL of distillated water.

The 6-TG/Au/NPs was synthesized by addition of 50 mL water solution containing 5 mg (17.5 µmol) of 6-TG to previous solution containing Au/NPs (30 s after sodium borohydride addition).

Drug loading efficiency (LE)

Using equation 1, we can determine the loading efficiency (LE) for 6-TG at an Au anoparticle surface using UV/Visible spectrophotometry data

6-TG loading efficacy (%)= (Weight of 6-TG at Au/NPs)/(Weight of 6-TG used in formulation) ×100 (1)

6-TG release profile from Au/NPs

The release profile of 6-TG from Au/NPs surface was obtained using dialysis. The investigations are same with Shakeri et al., reported procedure that is one of the authors of this paper[25]. 6-TG concentration was specified by UV spectrophotometry. Cumulative release rate of 6-TG can be calculated using equation (2):

Release= [(total 6-TG loaded on Au/NPs) – (remaining 6-TG at Au/NPs after releasing process)]/[total 6-TG loaded on Au/NPs] × 100 (2)

In vitro cytotoxicity studies

The cell cytotoxicity of the unloaded 6-TG, unloaded Au/NPs and 6-TG loaded Au nanoparticles was evaluated in MCF-7 by MTT assay. 2×10^4 cells/ml were seeded in 96-well culture plates containing 200 µl of medium and incubated for 24 h and subsequently the culture medium was replaced by fresh medium containing different formulations of nanoparticles and incubated for 48 h. The wells without drug and nanoparticle were used as control. After treatment, media were carefully removed and MTT (5 mg/ml) was added to each well. Microplates were incubated for 4 h at 37°C, and then the medium was removed. Then, absorbance was assessed at 490 nm using a microplate reader. Cell viability was calculated as below:

Cell viability% = (A exp − A neg)/(A con − A neg)

Where A exp is the absorbance of treated cells, A neg is the absorbance of blank, and A con is the absorbance of control cells.

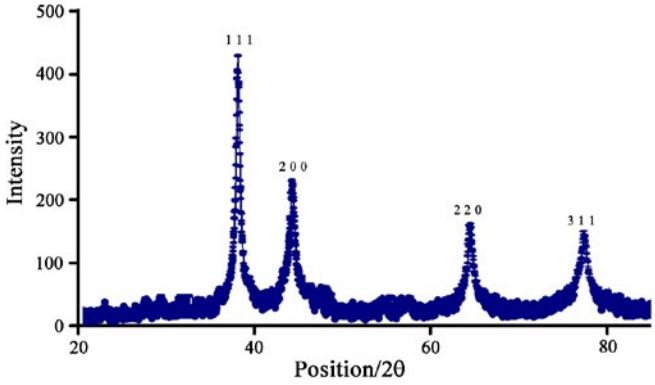

Fig. 1. XRD pattern of Au nanoparticles synthesized in this work.

RESULTS AND DISCUSSION

Characterization of Au nanoparticles

The Au nanoparticle morphology and structure was characterized by XRD, DLS and TEM methods. As can be seen in Fig. 1, the XRD pattern of Au nanoparticles showed planes with miller indexes (1 1 1), (2 0 0), (2 2 0) and (3 1 1) relative to Au nanoparticles with (JCPDS no.04-0784).

In addition, TEM method was used for morphological investigation of Au/NPs (Fig. 2). As can be seen, Au/NPs were synthesized in spherical shape with diameter ~ 5.0 nm. The DLS data for unloaded Au/NPs are similar to TEM data (Fig. 3 A) that confirms the synthesized of Au/NPs.

Characterization of 6-TG loaded Au/NPs

The Au nanoparticle loaded with 6-TG was characterized in the next step by DLS and UV-Vis spectroscopy methods. The Au nanoparticle loaded with 6-TG was characterized by DLS method in the first step. The Fig. 3 shows the DLS diagrams for unloaded (diagram a) and loaded Au nanoparticle

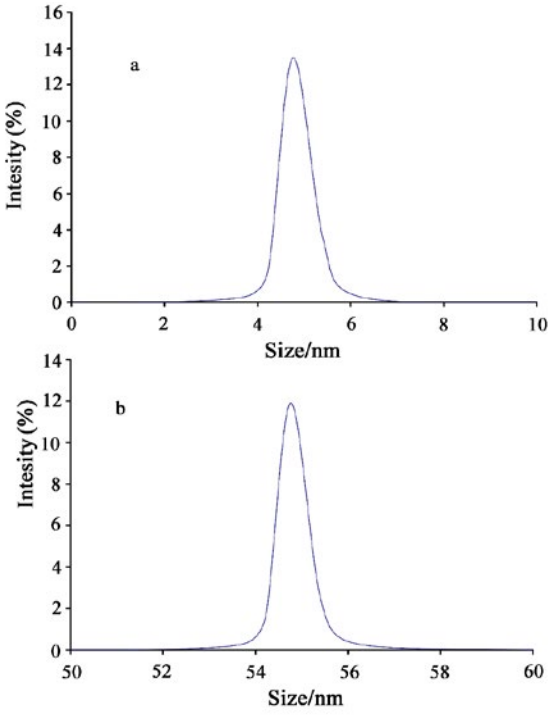

Fig. 2. TEM image of Au nanoparticles synthesized in this work.

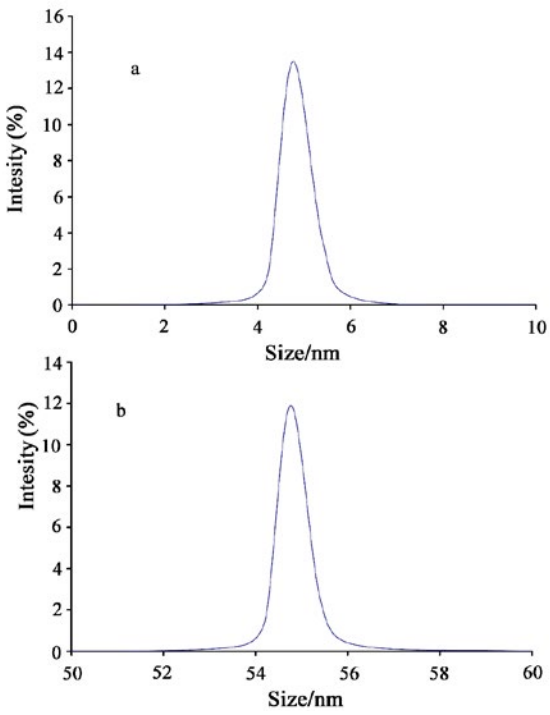

Fig. 3. DLS spectrums of (a) Au nanoparticles and (b) 6-TG loaded Au nanoparticles.

with 6-TG (diagram b). The comparing DLS data for unloaded Au/NPs (Fig. 3a) and 6-TG/Au/NPs (Fig. 3b) showed that the presence of 6-TG at a surface of Au/NPs increase the diameter of Au nanoparticles due to strong bod of Au-S. The increasing in Au nanoparticle diameter confirms loading of 6-TG at a surface of Au nanoparticle and synthesized of 6-TG/Au/NPs for biological application.

For more investigation, we recorded UV-Vis spectrums of Au^{3+} solution (as a precursor to the synthesis of gold nanoparticles), Au/NPs and 6-TG/Au/NPs at a same condition (Fig. 4). As can be seen, the Au^{+3} solution did not showed any significant absorbance bond in UV-Vis spectrum (curve a). After reducing of Au salt by sodium borohydride and formation of Au/NPs a strong absorbance bond was observed at a λ~550 nm that is relative to surface plasmon resonance of Au nanoparticles (curve c). After loading of Au/NPs with 6-TG drug, the intensity of absorbance bond for Au/NPs strongly reduced due to Au-S bond between Au nanoparticle and thiolic group of 6-TG and

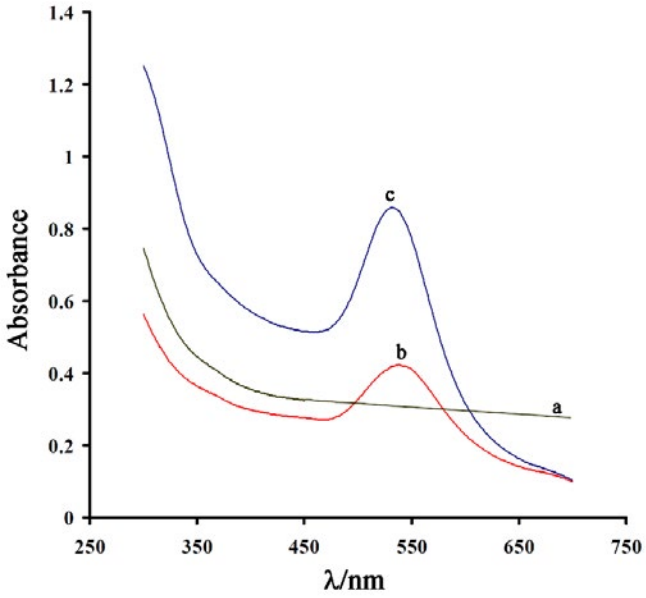

Fig. 4. UV-Vis spectrums of Au^{+3} (a); 6-TG loaded Au nanoparticles (b) and Au/NPs (c).

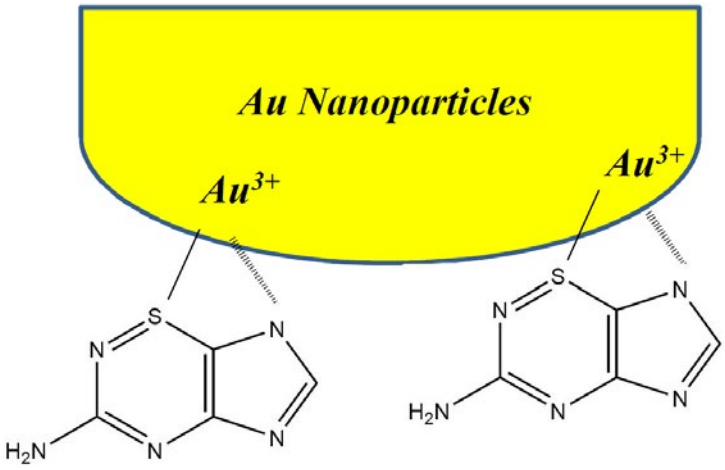

Fig. 5 Stability of 6-TG loaded Au/NPs over 15 days

deactivate Au/NPs surface (Fig. 5). According to the above data, we can concluded the synthesized of 6-TG/Au/NPs in nanoscale that can be useful for drug delivery systems.

The stability of the 6-TG/Au/NPs studied as an important factor for designing a novel DDSs. The synthesized 6-TG/Au/NPs placed in water for 15 days at 4°C and the samples analyzed by DLS method at predetermined times (Fig. 6). As can be seen, the diameter of 6-TG/Au/NPs size increased gradually from 54.76 to 55.5 nm and then sediment from solution.

Application of 6-TG loaded Au/NPs in DDS

According to Wang et al. report [26], the absorbance bond with a wavelength of 320 nm can be relative to 6-TG and was used this absorbance bond for the determination of 6-TG loading at a surface of Au nanoparticle. Using equation (2) and obtained data from UV-Vis spectrum, the LE% of 6-TG on Au/NPs was determined as 68.3%±3.9. The release profiles the 6-TG loaded Au/NPs at different time can be observed at Fig. 7. The 6-TG loaded Au/NPs release profile was monitored in RPMI 1640 medium and 100 rpm at 37°C for 120

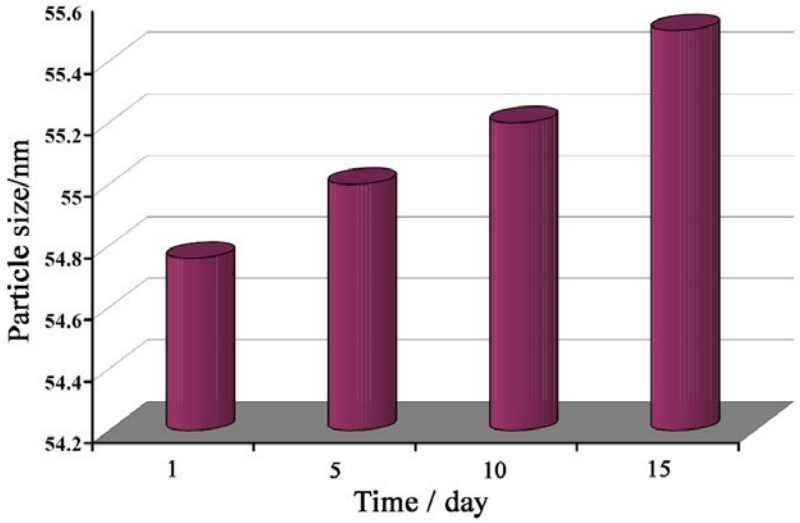

Fig. 6. The release profiles for 6-TG-loaded Au/NPs at the different time interval in RPMI medium

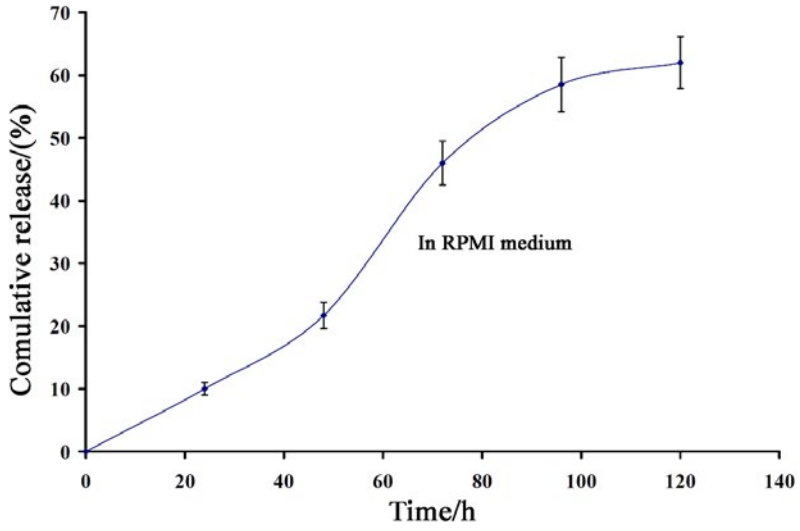

Fig. 7. Treatment of MCF-7 cell line with different concentrations (µg/ml) of free 6-TG.

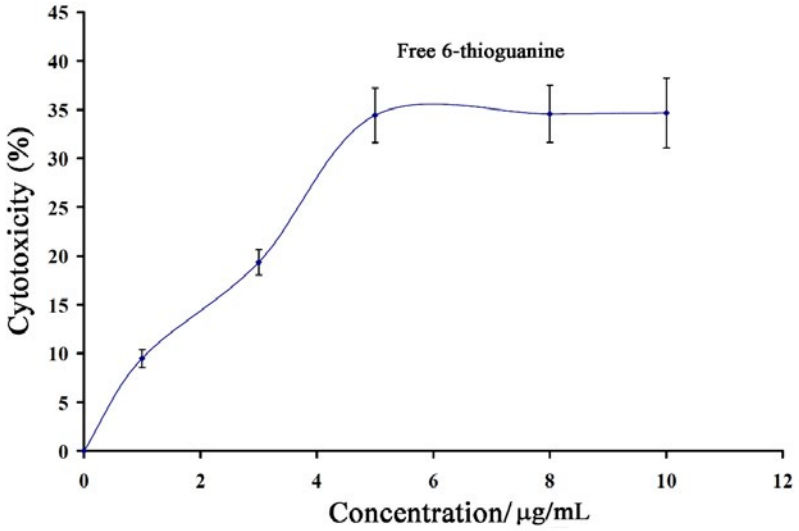

Fig. 8. Treatment of MCF-7 cell line with different concentrations (µg/ml) of unloaded Au/NPs and 6-TG-loaded Au/NPs.

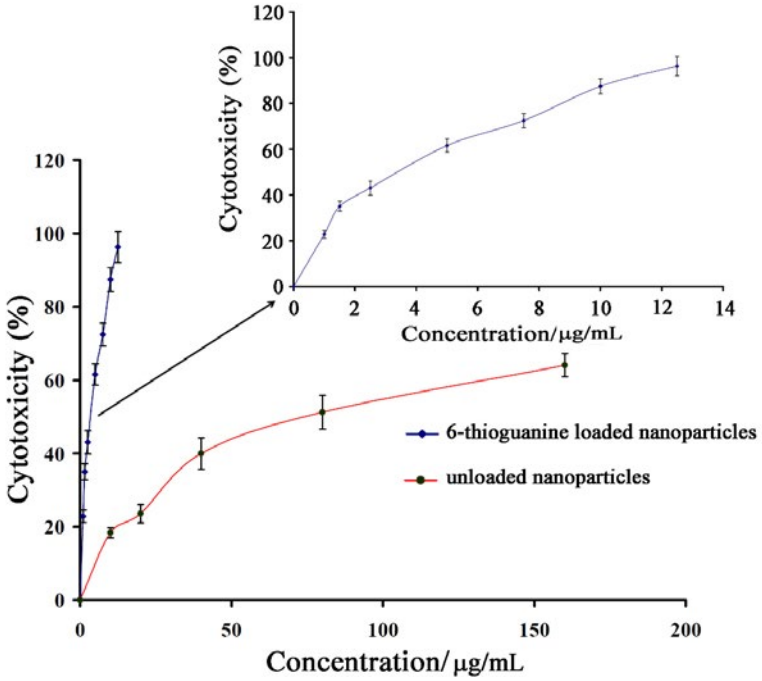

Fig. 9. IC_{50} values for unloaded Au/NPs, free 6-TG and 6-TG-loaded Au/NPs

h. 6-TG released from Au/NPs was 62.0% after 120 h of incubation that is a good condition for DDSs into the RPMI 1640. The most important time for assessment of drug releasing is the first 24 h of incubation. Initial burst release (IBR) of drug must be less than 10-15% of total entrapped drug. In this study, IBR is 10% and showed that the most amount of 6-TG is entrapped inside of HSA nanoparticles.

In vitro cytotoxicity studies

Cytotoxicity of free 6-TG, Au/NPs, and 6-TG/Au/NPs was investigated against breast cancer cells line (MCF-7) (Figs. 8 and 9). The recorded confirmed that 6-TG/Au/NPs have more cytotoxicity effect on the MCF-7 cells when compared with unloaded Au/NPs. According to these data, we found that 6-TG increases anticancer activity when loaded onto Au/NPs.

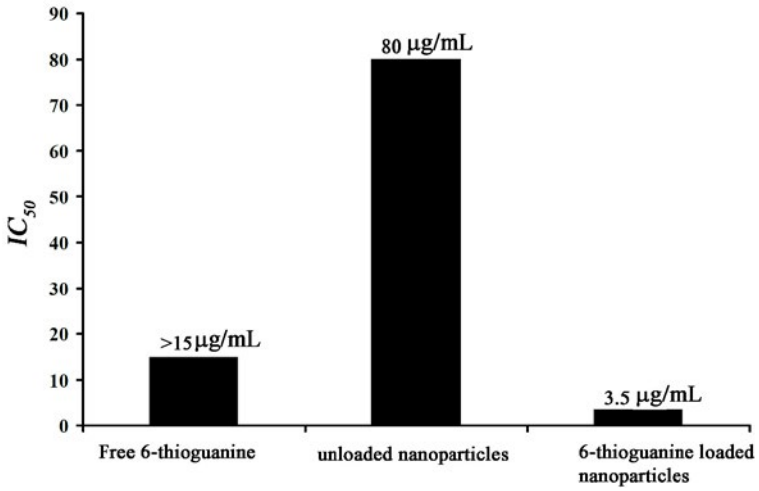

Fig. 10. Schematic of 6-TG stabilization of Au/NPs

IC_{50} for 6-TG, Au nanoparticles, and 6-TG/Au/NPs were >15.0, 80 and 3.5 µg/ml, respectively (Fig. 10). According to obtained data, -TG/Au/NPs demonstrated more cytotoxicity activity in the low concentration range when compared to Au/NPs or free 6-TG that can be relative to controlled release of 6-TG from the Au/NPs. The slow and controlled release rate of 6-TG from the Au/NPs demonstrates the biocompatibility of the prepared DDSs.

CONCLUSION

In this study, we suggested a new drug delivery systems based on loading of 6-thioguaine anticancer drug at as surface of Au nanoparticles. The Au nanoparticle well synthesized with diameter ~5 nm. The 6-TG/Au/NPs showed good cytotoxicity effect on breast cancer cells line (MCF-7). IC_{50} for 6-TG, Au nanoparticles, and 6-TG/Au/NPs were >15.0, 80 and 3.5 µg/ml, respectively.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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