Cytotoxicity evaluation of environmentally friendly synthesis Copper/Zinc bimetallic nanoparticles on MCF-7 cancer cells

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Abstract
Bimetallic nanoparticles offer unique chemical, physical and optical properties that are not available for monometallic nanoparticles. Bimetallic nanoparticles play a major role in various therapeutic, industrial and energy fields. Recently, nanoparticles of Copper/Zinc bimetallic nanoparticles have attracted attention in various fields, especially medicine. In this study, bimetallic CuO/ZnO nanostructures were biosynthesized using plant extracts. The plant-mediated synthesis nanoparticles were characterized by Transmission electron microscopy (TEM), X-ray diffraction analysis (XRD), Field Emission Scanning Electron Microscopy (FESEM) and Energy-Dispersive Spectroscopy (EDAX). The cytotoxicity of plant-mediated synthesis bimetallic nanoparticles and the synergistic effects of these nanoparticles in combination with the anticancer drug doxorubicin on MCF-7 cancer cells were evaluated by MTT assay.

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1 Introduction

Nanotechnology is a novel technology that has spread rapidly due to its transformative, miraculous and incredible effects (Cao et al. 2021a; Chu et al. 2021). Nanotechnology has wide applications in various fields from food (Ningthoujam et al. 2022; Parimaladevi et al. 2018), medicine (Chu et al. 2022), medical diagnostics (Nazari-Vanani et al. 2019) and biotechnology (Kannan et al. 2021; Rabiee et al. 2021) to electronics (Song et al. 2015), computers (Zhao et al. 2021), communications (Zhao, Khan 2021), transportation (Mathew et al. 2019), energy (Dey et al. 2022), environment (Kannan et al. 2020; Liu et al. 2022), materials (Cao et al. 2021b; Wang et al. 2022), aerospace (Haynes and Asmatulu 2013), and national security (Zha 2021). Currently, the special properties of these nanomaterials such as magnetic properties, quantum size, optical and biological properties have been considered by scientists (Luo et al. 2021; Wang et al. 2022; Rahdar et al. 2021a; Rahdar et al. 2021b). Nanomaterials are one of the promising areas of medical research and have been used to diagnostic and treatment of many dangerous diseases, including COVID-19 (Tang et al. 2021). Among the types of nanoparticles, bimetallic nanostructure from the combination of two different metals have unique properties in the field of light, heat, catalytic, drug delivery and therapeutic effects (Alijani et al. 2020). The physical and chemical properties of bimetallic and tri-metal nanoparticles are different from the properties of individual metals due to the compositions and interactions between the individual components of these nanostructures (Khatami et al. 2018; Wang et al. 2018). Nanoparticle compounds lead to the search and design of new nanostructure with unique properties that attract the attention of scientists in various fields of medicine and academia. On the other hand, due to drug resistance, inefficiency of current treatments, including side effects and high costs for the treatment of various infectious diseases, autoimmunity and cancer, researchers have turned to the use of nanoparticles in medical treatments (Gao et al. 2019; Zhang et al. 2022). In the meantime, the biosynthesis approach has been reported as an easy, environmentally friendly and cost-effective method for the synthesis of nanoparticles using plant extracts. Green synthesis of nanoparticles using plant extracts has some benefits such as lower cost, environmental friendliness and the possibility of cheap and easy production on an industrial scale. In addition, in green synthesis methods, unlike the main chemical methods, there is no need to use high temperature and pressure and several chemical compounds, so this approach as an economical, valuable and environmentally friendly method can be used to produce metal particles should be used on an industrial scale. In green synthesis of nanostructure using plant extracts, these extracts act as a reducing agent and also as a coating agent in the process of synthesis and stability of nanoparticles (Khatami et al. 2021).

Copper is a metallic element with high ductility and thermal and electrical conductivity (Peralta 1994). Copper is an essential mineral for living organs because it plays a key role in the production of the respiratory enzyme of cytochrome oxidase C (Kulandaivel et al. 2022; Stern 2010). Copper nanoparticles exhibit unique properties such as catalytic, antifungal (Asghar et al. 2018) and antibacterial activity (Parimaladevi et al. 2018) that are not found in commercial copper. Copper oxide nanoparticles with apoptotic effect (Chakraborty and Basu 2017) on cancer cells are desirable anticancer agents (Chakraborty and Basu 2017). Zinc is a rare trace mineral that has the highest levels in the body after iron (Allai et al. 2022). Zinc is found in Zinc Finger Proteins and enzymes such as Superoxide Dismutase (Kaneda-Nakashima et al. 2022; Khan and Malik 2022). Research shows that there is a direct relationship between the amount of zinc in the body and the inflammation of cells. Reducing the amount of zinc in the body stimulates immune cells. In this way, the lack of zinc in the body causes inflammation in the cells of the body. One of the severe side effects of zinc deficiency in the body is the stimulation of cancer cells and inflammation of some cells (Yang et al. 2017; Prasad 2008). Research has shown that zinc is an important therapeutic agent in the control and treatment of cancer, as high concentrations increase apoptosis in cancer cells. It is also known as a tissue and skin repairer and is widely used in cosmetics (Aflatoonian et al. 2017; Bisht and Rayamajhi 2016).

In this study, Copper/Zinc bimetallic nanostructures were biosynthesized using plant extracts. The characterization of the biosynthesized nanostructure was studied by XRD SEM EDS and TEM analyses. Finally, the anticancer properties of bimetallic nanoparticles synthesized against breast cancer cells were evaluated by MTT method.
2 Materials and methods

2.1 Preparation of Copper/Zinc bimetallic nanoparticles

Copper/Zinc bimetallic nanoparticles were produced with *Lonicera caprifolium* plant extract. 7 g of plant powder was mixed with 50 mL of deionized water (sterile) and stirred at 25 °C (24 h). It was then placed at 100 °C for 15 min. The prepared extract was finally filtered. 3.4 g of Zn(CH$_3$COO)$_2$ * 2H$_2$O (Merck, ≤ 100%) and 1.4 g of CuCl$_2$ * 2H$_2$O (Merck, ≥ 99%) were added to 200 mL of the extract at 80 °C and dissolved; then, the pH of the mixture was increased to 7.5 by adding NaOH (Merck) ≥ 99%). The reaction mixture was sterilized for 3 h at 80 °C. After washing with deionized water, it was dried at 80 °C and calcined at 500 °C for 5 h (Chu et al. 2022).

2.2 Characterization of Copper/Zinc bimetallic nanoparticles

The chemical composition and morphology of the Copper/Zinc bimetallic nanoparticles synthesized were evaluated using the Sigma VP microscope (ZEISS German). The Copper/Zinc bimetallic nanoparticles morphology was studied by TEM microscope Tecnai G2 Spirit (Twin; FEI, Czech Republic) at voltage of 120 kV. XRD analysis was performed to determine the crystal phase and crystal size of Copper/Zinc bimetallic nanoparticles using Panalytical Holland X’PertPro at an angle of $2\theta$ from 10° to 80°. This analysis was performed with a Cu Kα anode of 1.54 angstroms. The particle size was calculated using Debye–Scherrer formula:

$$D = \frac{K\lambda}{\beta \cos \theta}.$$  

In the Debye–Scherrer formula, the $K$ components are the crystal shape coefficient (0.9), $\lambda$ is the wavelength of X-ray (0.154 nm), $\theta$ is the diffraction angle, and the beta peak width at half of the maximum height.

2.3 MTT assay

MCF-7 cancer cells were prepared from Pasteur Institute in Tehran, Iran. Cells in Dulbecco’s Modified Eagle’s Medium (DMEM; GIBCO) medium containing 10% FBS; GIBCO and 1% penicillin/streptomycin antibiotic solution were cultured and stored at 37 °C and 5% carbon dioxide. After reaching a density of 90%, the cells were treated with different concentrations (0–600 µg/mL) of biogenic Copper/Zinc bimetallic. Then, the effect of inhibiting the growth of cancer cell line was measured by MTT method. The optical absorption of Formazan crystals after dissolution in DMSO: Dimethyl sulfoxide was recorded at 570 nm with the help of ELISA.

3 Results

Figure 1 shows the XRD spectrum of the resulting synthesized Copper/Zinc bimetallic nanoparticles. In the XRD spectrum, distinct and sharp peaks are observed in the range of 31.9, 34.5, 35.5, 36.3, 38.8, 47.7, 56.7, 63.01, 66.5 and 68.1 degrees. Peaks of 35.5, 38.8, 66.5 and 68.4 degrees correspond to (002), (111), (311) and (220) Plane of pure CuO nanoparticles, respectively (Cao et al. 2021b). This single-phase with monoclinic structure confirms copper oxide nanoparticles. The strongest peak is at $2\theta = 35.5$ degrees, which according to the Debye–Scherrer formula, the average crystallite size of copper oxide nanoparticles is below that 50 nm. Peaks of 31.9, 34.5, 36.3, 47.7, 56.7, and 63.01 degrees correspond to (100), (002), (101), (102), and (110)
planes of pure ZnO nanoparticles, respectively. This confirms the hexagonal wurtzite structure of zinc oxide nanoparticles (Bisht and Rayamajhi 2016). The strongest peak is at 2θ = 36.3 degrees, which according to the Debye–Scherrer formula, the average crystallite size of zinc oxide nanoparticles is below that 50 nm.

Figure 2 shows the results of the FESEM-EDS analysis. The surface morphology of bimetallic nanoparticles with a magnitude of 20.0 Kx is shown in Fig. 2a. Spherical like nanoparticles can be seen in the image. The synthesized biogenic nanoparticles contain zinc, copper, oxygen and carbon with weight percentages of 50.2, 16.7, 19.3 and 13.8 Wt%, respectively. (Fig. 2b) The presence of carbon element corresponds to the organic precursor present in the synthesis steps (plant extract).

Figure 3 shows a TEM micrograph (a) and size distribution chart (b) of Copper/Zinc bimetallic nanoparticles with a bright-field background. Different types of rod, spherical and polyhedral Copper/Zinc bimetallic nanoparticles with different orientations are shown. This is consistent with the SEM results. As can be clearly seen in the picture, in some areas, the spherical nanoparticles are placed in order.

The cytotoxic effects of Copper/Zinc bimetallic nanoparticles on MCF-7 cell line were analyzed using MTT assay. The cytotoxic effects of anticancer drug (doxorubicin) were studied. The cytotoxic effects activity of Copper/Zinc bimetallic nanoparticles on MCF-7 cell line were studied. The IC50 was about 54 µg/mL. These NPs showed toxic effects against MCF-7 cell line, and at a concentration of more 600 µg/mL, the cytotoxic effects reached 100% (Fig. 4).

4 Discussion

In this study, Copper/Zinc bimetallic nanostructures were synthesized and characterized using plant extracts. The cytotoxicity of bimetallic nanostructures synthesized alone and the synergistic effects of these nanoparticles in combination with the anticancer drug doxorubicin on MCF-7 cancer cells were evaluated. Among nanoparticles, the effect of monometallic nanoparticles in various fields, including medical and industrial, is less than that of bimetallic and multimetallic nanoparticles. At present, in the treatment of disease, monometallic nanoparticles do not achieve the desired result. Therefore, according to the desired purpose of combining monometallic nanoparticles, they increase the effectiveness of bimetallic and trimetallic nanoparticles produced (Ali et al. 2021). In this study, Copper/Zinc bimetallic nanostructures were biosynthesized using green chemistry. Its physicochemical properties were evaluated using XRD SEM EDS TEM analysis. The synthesized bimetallic nanoparticles are completely pure. The structure of zinc oxide wurtzite and single-phase copper oxide was confirmed by XRD analysis. Copper/Zinc bimetallic nanoparticles have particles with different morphologies from spherical to rod and polyhedral. Bar and polyhedral structures increase the volume to the surface area of nanoparticles and increase the impact and contact surface.

Some synthesis of bimetallic nanoparticles was reported previously. Ismail et al. synthesized bimetallic CuNi and CuAg nanoparticles using ginger powders (Ismail et al. 2018). Kumari et al. (2015) obtained Ag–Au bimetallic nanoparticles using pomegranate juice (Meena Kumari et al. 2015). Dobrucka et al. (2019) produced CuO–ZnO and
Au–CuO nanoparticles using Cnici benedicti. The synthesized CuO–ZnO and Au–CuO bimetallic nanoparticles were analyzed for antibacterial activity as well as their effect on cell viability, using two human brain glioma cell lines. The size of ZnO–CuO nanoparticles (28 nm) showed toxicity on brain cells, depending on the time and concentration. In the first stage, nanoparticles limited the ability of cell division. They then blocked the cell cycle (in the G2-M phase), eventually leading to cell death. Antimicrobial activity studies showed that CuO–Au nanoparticles inhibited the growth of tested microbes at lower concentrations than ZnO–CuO nanoparticles and both nanoparticles showed excellent lethality. Renata et al. (Dobrucka et al. 2021) reported the green synthesis of CuO/Au/ZnO nanostructures using Verbenae officinalis extract. The toxic effect of CuO/Au/ZnO nanostructures on Jurkat cell line (ATCC© TIB-152™) was evaluated. The results showed that CuO/Au/ZnO nanostructures have a strong toxic effect on the cell line.
Copper/Zinc bimetallic nanoparticles were synthesized and characterized using plant extracts. The cytotoxicity of bimetallic nanoparticles synthesized alone and the synergistic effects of these nanoparticles in combination with the anticancer drug doxorubicin on MCF-7 cancer cells were evaluated by MTT method.

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Author contributions All the authors have read and approved the final manuscript.

Declarations

Conflict of interest The authors confirm that the content of this article involves no competing interests.

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