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Protocols

In vitro evaluation of cytotoxicity, possible alteration of apoptotic regulatory proteins, and antibacterial activity of synthesized copper oxide nanoparticles

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ABSTRACT

Copper oxide nanoparticles (CuO-NPs) were synthesized using a urea-based thermal decomposition technique, and characterized using different techniques. X-ray diffraction (XRD) and Raman spectroscopy confirmed the phase purity and crystalline structure of CuO-NPs. The size of CuO-NPs was investigated using XRD and was confirmed *via* dynamic light scattering analysis. CuO-NPs showed an average diameter of ~20 nm. The possible cytotoxicity of CuO-NPs was evaluated in HT-29 and SW620 cancer cell lines. The median inhibitory concentration of CuO-NPs in HT-29 and SW-620 cells was 4.99 and 3.75 μg/mL, respectively. The underlying mechanism responsible for apoptosis in colon cancer cells after CuO-NP exposure has not been well understood. In this study, we investigated the possible mechanisms of induction of apoptosis *via* analysis of the expression of Bcl-2 and Bcl-xL proteins in HT-29 human colon cancer cells after CuO-NP exposure. Western blot assay showed downregulation of Bcl-2 and Bcl-xL protein expression after CuO-NP exposure. Our findings may aid in the understanding of the potential mechanisms responsible for induction of apoptosis owing to inhibition of Bcl-2 and Bcl-xL protein expression. Furthermore, the antibacterial activity assay showed that the synthesized CuO-NPs did not exert significant inhibitory effects against different gram-positive and gram-negative bacteria *in vitro*.

1. Introduction

Nanoparticles are the building blocks of nanomedicine and nanotechnology. In the field of medicine, nanobiotechnology has been used in various applications, such as detection, diagnosis, drug delivery, and treatment of various diseases, including different types of cancer [1–5]. The recent intensification toward the implication of bioreactive nanoparticles helps to open a new era in the connection of nanotechnology with biotechnology. Various studies have shown the potential applications of the novel molecular benign technologies for therapeutic and diagnostic purposes in the biological system by implication of nanotechnology [4,6].

Cancer is a leading cause of mortality worldwide. Despite the advances made toward the understanding of the molecular basis of cancer and the development of surgical procedures, chemotherapy, and radiotherapy, as well as the identification of several cancer biomarkers, the overall cancer death rate has not significantly improved since the last two decades [7]. The progress achieved in nanotechnology and nanobiotechnology has been widely considered as a novel and revolutionary paradigm shift for diagnosis and treatment of different types of cancer. The design and synthesis of different types of nanoparticle (NP) systems using various materials may provide a promising approach for the treatment of various cancer types [3,5,8]. Therefore, more efforts are required to aid in

Abbreviations: CuO-NPs, copper oxide nanoparticles; DLS, dynamic light scattering; PDI, polydispersity index; XRD, X-ray diffraction; HT-29, human colon cancer cell line; SW-620, human colon cancer cell line; Bcl-2, B-cell lymphoma 2; Bcl-xL, B-cell lymphoma-extra large; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; MTT reagent, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; *E. coli*, *Escherichia coli*; IC_{50}, half maximal inhibitory concentration; °C, degree celsius; μg, microgram; mL, milliliter; h, hour.

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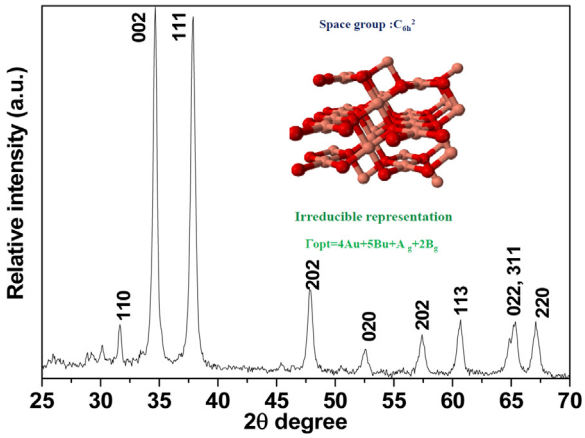

Fig. 1. XRD pattern of CuO-NPs with crystalline structure.

the development of new approaches that allow early diagnosis and treatment of cancer to increase the patient survival.

The size of nanoscale formulations ranges typically from 1 to 100 nm, which is equivalent to enormous biological molecules, including various enzymes, proteins, and receptors. NPs can offer specific interactions with biological molecules, and may be implicated in the treatment and diagnosis of different cancer types [4,9]. Copper oxide NPs (CuO-NPs) are used in paints, textiles, food, and plastics because of their significant antimicrobial properties [10,11]. CuO, with a band gap of ~1.7 eV, is the main p-type semiconductor [12]. It is widely used in various applications, including solar cells, gas sensors, and efficient temperature superconductors, as well as optical, catalytic, and various medical applications [13–19]. Numerous methods have been applied for the design and synthesis of CuO-NPs, including plasma process [20], sol-gel method [21], thermal decomposition method [22], polyol method [23], microwave-assisted method [24], wet chemical method [25], hydrothermal method [26], and solution combustion method [27]. These techniques involve the use of various hazardous compounds, high temperature, and pressure. It was found that these toxic chemicals may cause various adverse effects if absorbed on the surface of the NPs [28]. Various studies have shown the potential cytotoxicity of CuO-NPs in different cell lines, including human hepatocarcinoma (HepG2), human cardiac microvascular endothelial cells, human lung epithelial cells (A549), and neuronal cells [29–33]. However, few studies have investigated the toxicity and molecular mechanism of action of CuO-NPs in human colon cancer cells. Cancer growth and development is potentially regulated by programmed cell death (apoptosis) pathways. Uncontrolled proliferation, because of alterations or defects in different pathways, including the apoptotic pathways, is the most important characteristic of cancer cells [34].

Numerous genes are involved in the control and regulation of programmed cell death. Normal expression of Bcl-xL, Bcl-2, and p53 genes is considered the key regulator of the cell cycle [35]. These proteins coordinately regulate the cell cycle, DNA repair, and various apoptotic pathways; thus, they are involved in controlling and maintaining the genomic stability [35,36]. Bcl-2 and Bcl-xL are antiapoptotic proteins, which play key roles in the prevention of the mitochondria-dependent intrinsic cell death pathway, as well as the extrinsic cell death pathway. These antiapoptotic proteins have different roles in the inhibition of the intrinsic and extrinsic cell death pathways. Bcl-2 overexpression is capable of inhibiting apoptotic cell death; in addition, it inhibits nonapoptotic cell death by inducing cell cycle arrest in the G1 phase, which may result in cellular senescence [37,38]. The alteration in Bcl-2 expression may increase cell death with the interaction to Bcl-xL and other factors [39]. Various recent studies have shown that alteration and dysbiosis of the normal microbiota have also been involved in different chronic diseases, such as allergy and different cancers [40–43]. In addition, many studies revealed an association between different strains of bacteria and the growth and development of different cancer types. Besides the inflammatory response, increasing evidence suggests that perturbations in the intestinal microbiota are implicated in the growth and development of colorectal cancer [44,45]. The present study aimed to synthesize and characterize CuO-NPs. Furthermore, we investigated the potential cytotoxicity of the CuO-NPs in colon cancer cell lines using the MTT assay, as well as the underlying mechanisms *via* analysis of the expression of the antiapoptotic proteins, Bcl-2 and Bcl-xL in HT-29 colon cancer cell line. We also investigated the possible antibacterial activity of CuO-NPs against different gram-positive and gram-negative bacteria.

2. Experimental procedure

2.1. Chemicals and reagents

Copper nitrate, urea, dimethyl sulfoxide (DMSO), MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), and anti-β-actin antibody were purchased from Sigma-Aldrich. HT-29 and SW-620 colon cancer cell lines were obtained from the American Type Culture Collection (ATCC). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), Dulbecco phosphate-buffered saline, and antibiotics (penicillin and streptomycin) were purchased from Invitrogen Co., (Carlsbad, CA). Anti-Bcl-xL and anti-Bcl-2 primary antibodies, as well as the horseradish peroxidase-conjugated secondary antibodies, sodium dodecyl sulfate (SDS), and radioimmunoprecipitation assay buffer (RIPA buffer) were purchased from Santa Cruz Biotechnology, Inc, (Santa Cruz, CA). Other high-quality chemicals used in this study were purchased from commercial sources.

2.2. Synthesis of CuO-NPs

For the synthesis of CuO-NPs, copper nitrate and urea were used as starting precursors without any further purification. Copper nitrate (1 g) was dissolved in 50 mL of distilled water. Then, urea (2 g) was dissolved separately in distilled water and mixed with the copper nitrate solution with constant stirring at 100 °C for 2 h. The precipitate was separated *via* centrifugation, and properly washed with deionized water to remove the excess nitrates and amines. The precipitate was left to dry in air at 100 °C, and then annealed at 300 °C for 2 h.

2.3. Characterization of CuO-NPs

Various techniques were used to determine the physical properties of the synthesized CuO-NPs.

2.3.1. X-ray diffraction for determination of size of CuO-NPs

The crystallinity of the powder was evaluated by X-ray diffraction (XRD) technique at room temperature using a PANalytical X'Pert X-ray diffractometer equipped with a Ni filter using Cu-Ka (λ = 1.54056 Å) radiation as the X-ray source.

2.3.2. Raman spectroscopy

Raman spectra were recorded on a Jobin Yvon Horiba HR800 UV Raman microscope using a HeNe laser emitting at 488 nm.