Cytotoxic activity of green synthesis copper oxide nanoparticles using cordia myxa L. aqueous extract on some breast cancer cell lines.

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Abstract. The efficiency of copper oxide nanoparticles synthesized from aqueous extract of cordia myxa L, on some breast cancer lines AMJ-13,MCF-7, and HBL-100 as normal cell lines was studied. Different concentration of copper oxide nanoparticles (25,50,75,100) µg/ml at different times (24,48 and 72) h were selected. The results showed that the effect of nanoparticles depend on the concentration. As the concentration of nanoparticles increase the percent of inhibition increase. It was found that concentration of copper oxide nanoparticles at 100 µg/ml gave the highest inhibitory rate of cell growth MCF-7 (71.1%) after 24 hours, while the percent of inhibition for AMJ-13 was (69.6)%. When the exposure time was increased to 48 h, the rate of inhibition at 100 mg/ml was 80% for MCF-7 while 73% for AMJ13. By increasing the duration of the exposure to 72 hours, the rate of inhibition at 100µg/ml were (85.2 and 78.2) for MCF-7 and AMJ13 respectively. No significant inhibition was found for HBL-100 at different concentration and different times. These results was indicated that copper oxide nanoparticles synthesized from the aqueous extract of cordia myxa L. had a toxic effect on growth of some breast cancer cell lines.

Introduction

Cancer is the widespread disease and more than fifty percent of patients that diagnosed with cancer finally die from it. Cancer does not depend on age and sex. It comes second to cardiovascular disease leading cause of death in the world(1). Although there are many treatments such as chemotherapy, radiotherapy, and surgery but these treatment did not achieve the desired results. Therefore, the need for a suitable treatment for cancer was emphasized. One of the techniques that has been used to achieve the goal of cancer treatment is nanotechnology. Nanotechnology is known as the formulation, utilization and synthesis of materials at a scale up to 100 nm in diameter(2). The nanosize of particles change their physical and chemical properties and thus will lead to show various optical, magnetic and electric properties(3). The reason for the nanoparticles differ in their properties from conventional materials is the size, shape, distribution and surface / volume ratio of nanoparticles (4). Nanoparticles for metal oxides have attracted considerable attention to their potential applications in photovoltaics, nanoscale electronics, nanoscale sensors, nanoscale devices, information storage and stimulation (5). Copper oxide nanoparticles(CuONPs) is one of the most oxides widely used as a catalyst in the reduction process, optical stimulation and chemical reactions in the gas phase (6).
Different physical and chemical methods were used for synthesis of nanoparticles. These methods are hurtful because the chemicals used are toxic, flammable, and are not deducted easily to the environment (7). Biological synthesis of nanoparticles are the alternative methods to the conventional physical and chemical methods. The green methods have received great attention because of using clean nontoxic chemicals, eco-friendly solvents and synthesized nanoparticles in one step procedure (8). Plants are better synthesizers when compared to the other forms of biological sources (9). They supply nontoxic chemicals and provide natural capping agents. Moreover the use of plant also reduces the cost of microorganism isolation, culture media and the cost for the synthesis of nanoparticles (10). Cordia myxa L. is a species of flowering plant in the borage family, Boraginaceae. The plant was regional in Asia tropica and was introduced long ago in tropical Africa and Australia (11). Pharmacological studies revealed that Cordia myxa possessed analgesic, anti-inflammatory, immunomodulatory, antimicrobial, antiparasitic, insecticidal. In addition it has cardiovascular, respiratory, gastrointestinal and protective effects (12). In the present work, green synthesized copper oxide nanoparticles using cordia myxa L. was investigated against some breast cancer cell lines.

Materials and Methods

Synthesis of copper oxide nanoparticles.

Stock concentration of CuONPs concentration were synthesized from the leaves of cordia myxa L. as in the previous research (13). Briefly 3 ml 40 mM CuSO4·5H2O solution was added to the heated cordia myxa L plant extract and left at room temperature overnight. The change in color from brown to green was indicated the formation of copper oxide nanoparticles. The synthesized nanoparticles were characterized by UV-visible spectrophotometer, FTIR, X-ray diffraction, scanning electron microscope and atomic force microscope. The nanoparticles were sterilized using a filter with holes of 0.22 micrometer diameter. Four concentrations were prepared (100, 75, 50, 25) μg / ml using serum-free medium.

Breast cancer cell lines and cytotoxic studies

• The human breast adenocarcinoma (MCF-7) was developed in 1970 and the sample is taken from the breast tissue of a Caucasian woman aged 69 years.

• Infiltrating ductal carcinoma (AMJ13) The cells of this line are biopsied by a 70 year-old Iraqi woman. This line was developed at the Iraqi Center for Cancer Research and Medical Genetics (14).

• The epithelial cell line (HBL-100), non-tumoral epithelial cells.

All cell lines were obtained from the cellular bank unit of the Iraqi Center for Cancer Research and Medical Genetics. These cell lines were maintained in DMEM culture medium (Biochrom, Germany) supplemented with 10% fetal bovine serum (FBS; Biochrom, Germany), penicillin (100 U/mL) and streptomycin sulphate (100 mg/mL) at 37°C in 5% CO2. Cytotoxicity determinations are based on cellular staining with crystal violet. A volume of 200 μl of a cell suspension was plated in triplicate into 96-well microliter plates. Twenty-four hours later, cells were treated with the CuONPs at four dilutions (25, 50, 75, and 100) g / ml and exposed continuously for the next 24, 48 and 72 h. At the end of each exposure time the medium was removed and the cells were then stained with crystal violet and the optical density (OD) was measured at λ = 492 nm with a plate reader. The percentage of tumor inhibitory rate (IR%) was calculated according to the equation indicated in (15).

\[
\text{IR\%} = \frac{A - B}{A} \times 100
\]

where A = Absorbance of control (untreated cells), B= Absorbance of treated cells.
Statistical analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). Two-way ANOVA and Least significant differences (LSD) post hoc test were performed to assess significant differences among means. P < 0.05 was considered statistically significant (16).

Results

MCF-7, AMJ13 breast cancer cell lines and HBL-100 as normal cells were used to screen for the in vitro cytotoxic activity of copper oxide nanoparticles.

Effect of copper oxide nanoparticles on MCF-7 cell line:

In this study, toxic responses of copper oxide nanoparticles to human breast epithelial MCF-7 cells were investigated. After 24 h, the first concentration revealed higher significant inhibition P < 0.05 in comparison with the other concentration of CuONPs. No significant inhibition among the 50 and 75 µg/ml concentrations. By increasing the concentration from 25 to 100 µg/ml the rate of inhibition increase from 35.57 % to 71.18 %. By increasing exposure time to 48 h the first concentration revealed higher significant inhibition P < 0.05 in comparison with the other concentration. No significant inhibition among the 50 and 75 µg/ml concentrations. By increasing the concentration of CuONPs from 25 to 100 µg/ml the rate of inhibition increase from 49.41 % to 80.6 %. While at 72h incubation period, the first concentration revealed higher significant inhibition in comparison with the other concentrations except the second concentration. No significant inhibition among the 75 and 100 µg/ml concentrations. By increasing the concentration from 25 to 100 µg/ml the rate of inhibition increase from 57.49 % to 75.25 %. The maximum rate of inhibition of MCF-7 cell line at 100 µg/ml was 80.60 % after 48 h time exposure (Table 1).

| Table (1) cytotoxic effect of different concentration of copper oxide nanoparticles at different time exposure toward MCF-7 cell line. |
|---|---|---|---|---|
| Time | 25 µg/ml | 50 µg/ml | 75 µg/ml | 100 µg/ml |
| 24 h | 35.57±2.63 | 55.25±0.94 | 62.09±1.30 | 71.18±1.72 |
| C c | B b | B b | A a |
| 48 h | 49.41±1.48 | 65.68±2.54 | 70.62±2.39 | 80.60±1.46 |
| C b | B a | B a | A a |
| 72 h | 57.94±1.40 | 65.49±3.64 | 71.87±3.19 | 75.25±6.77 |
| B a | AB a | A a | A ab |
| LSD | 8.4424 | | | |

The results represent mean±SD, Capital letters within a single row represent a comparison between the concentrations and small letters within a single column representing the comparison of times.

Effect of copper oxide nanoparticles on AMJ13 cell line:

After 24 h, a significant inhibition showed between all concentrations P < 0.05. By increasing the concentration of CuONPs from 25 to 100 µg/ml the rate of inhibition increase from 26.37 % to 69.63 %. By increasing exposure time to 48 h the first concentration revealed higher significant inhibition P < 0.05 in comparison with the other concentration. By increasing the concentration from 25 to 100 µg/ml the rate of inhibition increase from 33.05 % to 73.73 %. While at 72h incubation period, the first concentration revealed higher significant inhibition in comparison with the other concentrations, but no significant inhibition between 75 and 100µg/ml concentration (Table 2). The cell toxicity observed after 72 hours of exposure at the concentrations of 25, 50, 75, and 100 mg/mL was 39.86%,
54.59%, 72.6%, and 78.36% respectively. The maximum inhibition of AMJ13 cell line at 100 µg/ml was 78.26 after 72 hours.

Table (2) cytotoxic effect of different concentration of copper oxide nanoparticles at different time exposure toward AMJ13 cell line.

| Time | 25 µg/ml | 50 µg/ml | 75 µg/ml | 100 µg/ml |
|------|----------|----------|----------|-----------|
| 24 h | 26.37±0.84 | 38.98±4.56 | 51.80±1.51 | 69.63±1.65 |
| 48 h | 33.05±4.76 | 43.44±2.48 | 63.59±2.22 | 73.73±5.05 |
| 72 h | 39.86±2.82 | 54.59±2.04 | 72.60±2.63 | 78.26±2.24 |

The results represent mean±SD, Capital letters within a single row represent a comparison between the concentrations and small letters within a single column representing the comparison of times.

Effect of copper oxide nanoparticles on HBL-100 cell line:

As shown in table 3 there was no significant inhibition by increasing in the concentration of CuONPs at the same time. In addition there was no significant inhibition by increasing time exposure for the same concentration.

Table (3) cytotoxic effect of different concentration of copper oxide nanoparticles at different time exposure toward HBL-100 cell line.

| Time | 25µg/ml | 50µg/ml | 75µg/ml | 100 µg/ml |
|------|---------|---------|---------|-----------|
| 24h  | 3.55±0.46 | 3.40±0.61 | 4.41±0.61 | 4.49±0.51 |
| 48h  | 3.92±0.42 | 3.37±0.65 | 3.55±0.49 | 4.02±0.33 |
| 72h  | 4.20±0.97 | 3.53±0.56 | 3.83±0.84 | 4.66±0.51 |
| LSD  | 1.7809   |         |         |           |

The results represent mean±SD.

Discussion

Several factors affect the inhibition rate of cancer cell lines such as the type of cell line, exposure period and concentration of the treatment used. But, when using nanoparticles as an inhibitor growth of cancer cells, it also depends on the morphology, size, surface area and capping groups (17). Our results shown that MCF-7 cell line was more effect than AMJ13 cell line when exposed to CuONPs at the same time and at different concentration, more over cancer cell lines more sensitive to nanoparticles than normal cells. This means that copper oxide nanoparticles have a selective effect on cells. Maryam et al were found that copper nanoparticles showed the ability to inhibit the growth of cancer cells (K562) and non-effect on the normal cell line (PBMCs) (18). In another study the HepG2 cells were exposed to CuONPs at different concentrations and showed that CuONPs significantly decreased the cell viability in dose dependent manner (19).

The selectivity of some metal oxide nanoparticles was studied like iron oxide nanoparticles and zinc oxide nanoparticles. In 2013, the study by Ahmed and his group showed that iron oxide nanoparticles had a selective effect on inhibition of (hepG2) and (A549) cell growth while there was no effect on inhibition of normal cell line growth (IMR-90)(20). Zinc oxide nanoparticles had a selective effect on inhibition of HL60 cell growth while there was no effect on inhibition of normal cell line growth (PBMCs)(21). Another study reported that zinc oxide nanoparticles kill human myeloblastic leukemia cells and are less toxic to normal peripheral blood mononuclear cells(22).
Along time ago copper compounds have been used to cure several diseases like cancer (23). Copper is recognized as a heavy metal that is toxic to mammalian cells, but with the rapid progression of nanotechnology, the NPs can target specific cells with minimum side effects. However the copper compounds have strong curative capacity(24). The toxicity of CuONPs on some cancer cell lines is due to the fact that copper ion Cu$^{2+}$ is released from nanoparticles and then binds to DNA in the cell, leading to DNA damage and thus cell death(25). The mechanism CuONPs was demonstrated through the induction of apoptosis with enhanced ROS generation(26). Apoptosis is a cell suicide mechanism that controls cell numbers. It can be induced through extrinsic and intrinsic pathways(27). Apoptosis process characterized by the morphological and biochemical changes and apoptosis of different cells in the same tissue does not occur at the same time (28).

This study suggest that CuONPs have ability to inhibit the growth of cancer cells in different types of breast cancer cell lines, and the inhibition was different according to type of cell line with less effect on normal cells.

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