J Nanostruct 14(1): 73-81, Winter 2024

RESEARCH PAPER

Anti-tumor Activity of Gold oxide Nanoparticles Synthesized Using *Nocardia Asteroids*

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ARTICLE INFO

Article History:
Received 12 October 2023
Accepted 24 December 2023
Published 01 January 2024

Keywords:
HepG2 cell line
MCF-7 cell line
MEF normal cell line
Nocardia bacteria
Tumor

ABSTRACT

During the current investigation in the field of clinical research, efforts were made to synthesize gold oxide nanoparticles (Au_2O_3NPs) and to explore their potential use in combatting breast cancer cell lines (MCF-10) and liver cancer (HepG2). To achieve this, *Nocardia* bacteria, isolated from soil, were utilized. An extract was prepared by mixing equal volumes of the bacterial extract and gold tetrachloride, resulting in the creation of gold oxide nanoparticles (Au_2O_3NPs). The formation of NPs was ascertained using ultraviolet-visible spectroscopy (UV-VIS). Characterization of the synthesized Au_2O_3NPs was conducted through various techniques including transmission electron microscopy, scanning electron microscope, atomic force spectroscopy, and X-ray diffraction. After the synthesis and characterization, the cytotoxicity of the gold oxide nanoparticles was evaluated against human breast cancer cells MCF-7, human liver cancer cells HepG2, and normal mouse embryo cells MEF, using varying concentrations (100, 75, 50, 25, 12.5, 6.25 micrograms/ml). The data demonstrated a concentration-dependent activity of the particles against the mentioned cells, with the half-cell inhibitory concentration (IC_{50}) of gold oxide nanoparticles derived from the *Nocardia asteroides* MT355849 indicating values of 46.84, 12.22, and 85.91 micrograms/ml against MEF, MCF-7, and HepG2 cell lines, respectively. Notably, the toxic effectiveness of the gold oxide nanoparticles on liver cells was found to be higher compared to breast cancer cells and normal cells. The results were further supported by statistical analysis performed using the Graph Pad Prism program, confirming the presence of significant differences between the inhibitory concentrations of cancer cells at a significance level of 0.05.

How to cite this article
Hassan F., Hateet R R., Al- Shakban M. Anti-tumor Activity of Gold oxide Nanoparticles Synthesized Using Nocardia Asteroids. J Nanostruct, 2024; 14(1):73-81. DOI: 10.22052/JNS.2024.01.007

INTRODUCTION

In the modern era, the term cancer is commonly used to describe a collection of diseases that are characterized by the abnormal and uncontrolled growth of cells [1, 2]. Genetic alterations that regulate the growth and division of cells can either be inherited or caused by exposure to environmental toxins during an individual's lifetime, resulting in physical changes [3, 4]. The development of cancer can be summarized into four distinct stages: cancer initiation, tumor dissemination, metastasis to distant organs, and resistance to chemotherapy [5, 6]. Cancer can affect various organs in the body, including the

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brain, lungs, liver, and breasts. Among women worldwide, breast cancer is the most prevalent type of cancer, accounting for 15% of cancer-related deaths. Although rare, breast cancer can also occur in males [7]. Cancer can manifest in various parts of the body and encompasses different types such as breast cancer, lung cancer, skin cancer, and colorectal cancer. The treatment of cancer necessitates collaborative efforts from specialized medical teams and researchers in the field of medical sciences. Common treatment options for cancer include surgery, radiotherapy, chemotherapy and targeted therapy [8]. Scientific studies indicate that cancer caused the deaths of approximately 8.8 million people in 2015 and nearly 10 million people in 2020 [9].

While mutations in DNA are the primary cause of cancer, two other significant factors contribute to its occurrence. The first factor is genetic predisposition, where certain inherited traits increase the likelihood of developing cancer. This genetic factor is estimated to account for 5-10% of cancer cases. The second factor is environmental factors, including exposure to carcinogenic substances and a combination of factors that contribute to an estimated 90-95% of cancer cases. These environmental factors encompass smoking, exposure to harmful ultraviolet rays, environmental pollution, malnutrition, occupational hazards, and more [10, 11]. Breast cancer is a form of cancer that originates in the lining cells of the breast or spreads to the axillary lymph nodes [12]. Typically, breast cancer develops within the ducts and lobes of the breast or other areas within it [13]. The primary cause of death related to breast cancer is the dissemination of cancer cells to distant parts of the body. This dissemination, known as metastasis, refers to the process in which cancer cells migrate from the original tumor to other locations within the body. Metastasis occurs when cancer cells enter the bloodstream or lymphatic system and travel to different areas away from the initial tumor site. Upon reaching a new location, these cells may form secondary tumors called metastatic tumors. The spread of cancer can worsen the condition and pose challenges for treatment [14].

Breast cancer is recognized as the most prevalent type of cancer and affects both genders equally. Among women worldwide, breast cancer ranks as the second leading cause of cancer-related deaths [15]. Liver cancer ranks as the fourth leading cause of cancer-related deaths worldwide [16]. The incidence and mortality rates of liver cancer are experiencing significant increases [17]. It is estimated that by 2025, over one million individuals will be affected by primary liver cancer each year, presenting a substantial risk, a significant health challenge, and a burden on society [18]. The most prevalent types of liver cancer are hepatocellular carcinoma (HCC), accounting for up to 90% of cases, and bile duct cancer (CCA), representing 10-15% [19]. Cancer treatment includes surgery, radiotherapy, immunotherapy, and chemotherapy. Chemotherapy is used in more than 50% of cancer cases as standard treatment [20]. However, it has some disadvantages such as the low effectiveness of targeted drug delivery to cancer cells and its effect on healthy cells [21]. Leading to side effects such as fatigue, hair loss, nausea, vomiting, and inflammation. Higher doses are used to achieve the desired drug concentration in cancer cells, which increases side effects [22].

Nanomedicine has been defined as the use of nanotechnology to treat, diagnose and monitor biological systems. Scientists are focusing on researching ways to deliver and target pharmaceutical, therapeutic, and diagnostic agents using nanotechnology. Nanomedicine has advanced in the field of cancer treatment to allow the identification of precise targets for specific clinical cases and the selection of appropriate drugs for nanosystems to achieve the desired response and reduce the side effects of anti-cancer drugs. This development means that nanotechnology and nanomedicine methodologies are designed and expanded for the benefit of humanity, contributing to improving the effectiveness and safety of cancer treatment. This work is part of ongoing efforts to develop treatment techniques and achieve progress in the field of cancer control [23,46,48-50]. The conventional process of encapsulating drugs within nanoparticles (NPs) offers several advantages. When drugs are encapsulated within NPs, drug half-life is increased and absorption across the cell membrane is improved, as well as controlled release of therapeutic substances at the target site. Thanks to the small size and distinct coating of NPs, they can easily carry and deliver hydrophobic anticancer drugs to the target site in the body, reducing their modification by the immune system [24].

The use of gold particles and their compounds (Au-NPs) is among the nanoparticles, based on

their distinctive properties that make them ideal for biomedical applications. Features such as chemical inertness and resistance to surface oxidation make Au-NPs ideal candidates for use in nanosynthesis. In addition, Au-NPs offer high stability, low cytotoxicity, biocompatibility, and multifunctional capabilities, making them a potential candidate for drug delivery systems [25]. Gold nanoparticles and their compounds cause toxicity to cancer cells through multiple mechanisms. These include the production of free radicals, glutathione oxidation, disruption of the cell cycle, activation of caspase 3, erosion of cancer cells, and programming cell death [26]. The study aimed to cytotoxicity study of laboratory-prepared gold oxide nanoparticles on two types of cancer lines: MCF7 breast cancer cells and HepG2 liver cancer cells and comparison with normal cells MEF.

MATERIALS AND METHODS

Applications of Synthetic Au_2O_3 NPs Nanoparticles on Tumour Cell Lines

Bacteria were isolated and identified according to our previous work [27], and the preparation of gold oxide nanoparticles followed the same protocol published previously [27]. Two cancer cell lines were selected, a liver cancer line (HepG2) and a breast cancer line (MCF-7). Breast and liver cells and normal cells were obtained from the Biotechnology Bank Unit in Baghdad Governorate in Iraq. Then, they were compared with the cells of the normal line MEF, which is the normal line of the mouse embryo for the growth of cancer cells following the method stated in [28]. The cells from each line were placed in a 25 cm^2 culture vessel containing RBMI-16401 culture medium and 10% bovine serum B. The plate, containing the cell suspension and culture medium, was incubated in a 5% CO_2 incubator at a temperature of 37°C for 24 hrs. After incubation, once it was confirmed that the cell culture exhibited growth and was free from contamination, a secondary culture was performed. Then, the cells were examined under an inverted microscope to ensure their viability, absence of contamination, and growth to reach the desired number of approximately 500 to 800 thousand cells/ml. Afterwards, the cells were transferred to a growth chamber, and the used culture medium was discarded. The cells were washed with a PBS solution, and discarded, and the process was repeated twice for 10 minutes each time. Then, an adequate amount of trypsin/ fersin enzyme was added to the cells and incubated at 37°C for 30-60 seconds, monitoring the transformation from a cell monolayer to single cells. The enzyme was then neutralized by adding a new culture medium containing serum. The cells were collected in centrifuge tubes and subjected to centrifugation at 2000 rpm for 10 minutes at room temperature to separate the cells from the trypsin and use a culture medium. The filter was discarded, and the cells were suspended in a fresh culture medium containing 10% serum. The cell count was determined by taking a specific volume of the cell suspension and adding an equal volume of trypan blue dye. The number of cells and their viability were assessed using a hemocytometer slide according to the equation:

$$C = N \times 10^4 \times F \backslash m$$

where C represents the number of cells in one ml of solution, N represents the number of cells on the slide, and F represents the dilution factor. The slide dimensions were 104. The percentage of cell viability in the sample was also calculated using the hemocytometer slide according to the equation Live cell viability = (live cells) \ (dead cells) x 100. The suspended cells were distributed into new vessels and then incubated in a 5% CO_2 incubator at a temperature of 37°C for 24 hrs.

Cytotoxic Assay of Nana Extracted on Cancer Cell Lines

Concentrations of the substance, namely 100, 75, 50, 25, 12.5, and 6.25 µg/ml, were prepared and sterilized using a 0.22-µ filter under sterile conditions. All prepared concentrations were used immediately after preparation. To prepare the cell suspension, the contents of a 25 cm^2 tissue culture bottle were treated with a trypsin/ fersin solution. After removing the old culture medium and gently stirring the bottle, it was incubated in a 37°C incubator for 10 minutes. Then, 20 ml of culture medium containing serum was added and mixed well with the cell suspension. Using a fine automatic pipette, 0.2 ml of the mixture was transferred to each well of a flat-bottomed microtiter plate for tissue culture. The plate was placed in the incubator at 37°C for 24 hours to allow the cells to adhere to the wells. After discarding the old culture medium, 0.2 ml of the previously prepared concentrations was added to each well, with three replicates for each

concentration. Additionally, three replicates were made for the control (cell suspension only). The plates were then incubated at 37°C. After the specified incubation time of 24 hours, the plate was removed from the incubator. A solution of crystal violet dye was added to all the wells containing cells, at a volume of 100 microliters per well. The plate was returned to the incubator for 20 minutes and then taken out. The contents were removed, and the cells were washed with water to remove excess dye. Live cells would take up the dye, while dead cells would not. The results were measured using an ELISA device at a wavelength of 492 nm. The percentage of cell inhibition was calculated using the following equation:

Percentage of cell inhibition = (absorbance reading of control cells - absorbance reading of treated cells for each concentration) / absorbance reading of control cells × 100.

statistical analysis

The obtained results underwent statistical analysis using the Graph Pad Prism version 6 analysis system. The means were compared using the Duncan Multiplex experiment to identify any significant differences, with a significance level set at $P \geq 0.05$.

RESULTS AND DISCUSSION

A test method [28] was used to evaluate the toxicity of different concentrations of gold oxide nanoparticles against HepG2 liver cancer cells

Table 1. Results of statistical analysis of liver cancer cell line concentrations

Standard deviation. inhibition ratio100%	Con. µg/ml
1.0 ± 21.17 f	6.25
1.1 ± 33.65 e	12.5
1.2 ± 35.70 d	25
1.2 ± 52.34 c	50
1.3 ± 60.29 b	75
2.1 ± 89.00 a	100

Different letters in the same column indicate statistically significant differences at the level (0.05≥P).

Table 2. Results of statistical analysis of breast cancer cell line concentrations

Standard deviation. inhibition ratio100%	Con. µg/ml
1.0 ± 35.82 d	6.25
1.2 ± 53.22 c	12.5
1.3 ± 65.92 b	25
2.1 ± 67.94 b	50
2.1 ± 77.66 a	75
2.3 ± 78.20 a	100

Table 3. Results of statistical analysis of normal MEF cell line concentrations

Standard deviation. inhibition ratio100%	Con. µg/ml
1.0 ± 05.12 e	6.25
1.2 ± 15.10 d	12.5
1.3 ± 25.02 c	25
1.2 ± 37.96 b	50
1.2 ± 47.76 a	75
1.3 ± 51.34 a	100

(Table 1), MCF-7 breast cancer cells (Table 2) and normal mouse embryo MEF cell lines (Table 3). Biosynthesized gold oxide nanoparticles from the filtrate of the bacterium *Nocardia asteroides* were used to evaluate anticancer activity. Six different concentrations of gold oxide nanoparticles were used in each sample, namely (100, 75, 50, 25, 12.5, 6.25) µg /ml, and measurements of the inhibition rates in MCF7 cells were repeated under the influence of different concentrations of nanoparticles for 24 hrs exposure at of 37°C for three replicates. The gold oxide nanoparticles had

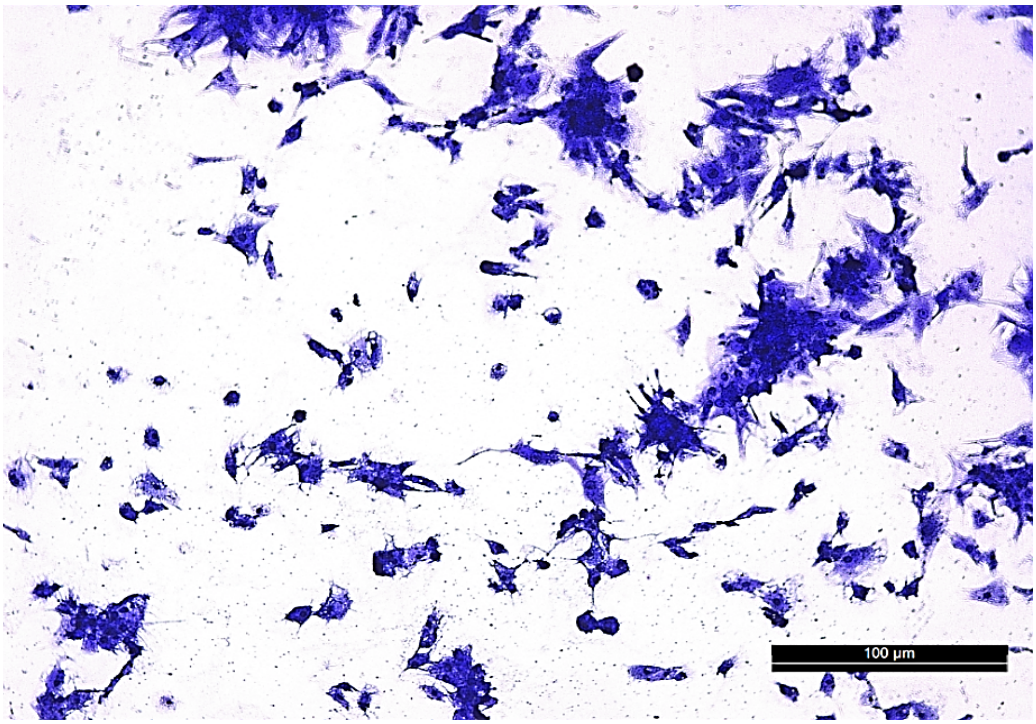

Fig. 1. The MCF7 cell line was used with a concentration of 100 µg of gold oxide nanoparticles dyed with crystal violet dye.

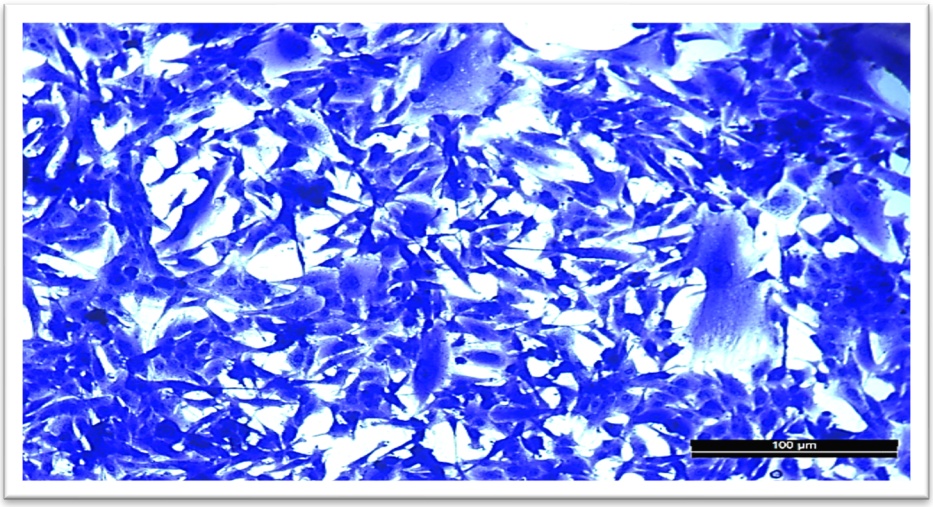

Fig. 2. HepG2 cell line at a concentration of 100, not treated with gold oxide nanoparticles and stained with crystal violet dye.

an inhibitory effect on the growth of cancer cells of the MCF-7 line, starting with a concentration of 6.25 micrograms/ml, where the inhibition rate reached 21.17%, and this percentage increased to 33.65%. And 35.70%, 52.34%, 60.29% and 89.00% for concentrations of 12.5, 25, 50, 75, and 100 µg/ml (Fig. 1), respectively. The reported results indicate that the toxicity of biosynthesized gold nanoparticles increases with increasing concentration.

This result is consistent with a study [29], which demonstrated that gold nanoparticles derived from mushrooms can inhibit breast cancer cells based on their concentration. It was documented in a study [30] that the PC-3 cell line showed significant cell death when exposed to high concentrations of gold nanoparticles (AuNPS). A study [31] found that different concentrations of gold nanoparticles (AuNPs) caused toxic effects on other cell lines and indicated that biosynthesized AuNPs were more cytotoxic than those that were chemically synthesized. The results of the statistical analysis confirmed the presence of significant differences between the different concentrations.

Liver cancer cells that were not treated with gold nanoparticles are illustrated in Fig. 2, however, gold oxide nanoparticles had an inhibitory effect (Fig. 3) starting from a concentration of 6.25, where the

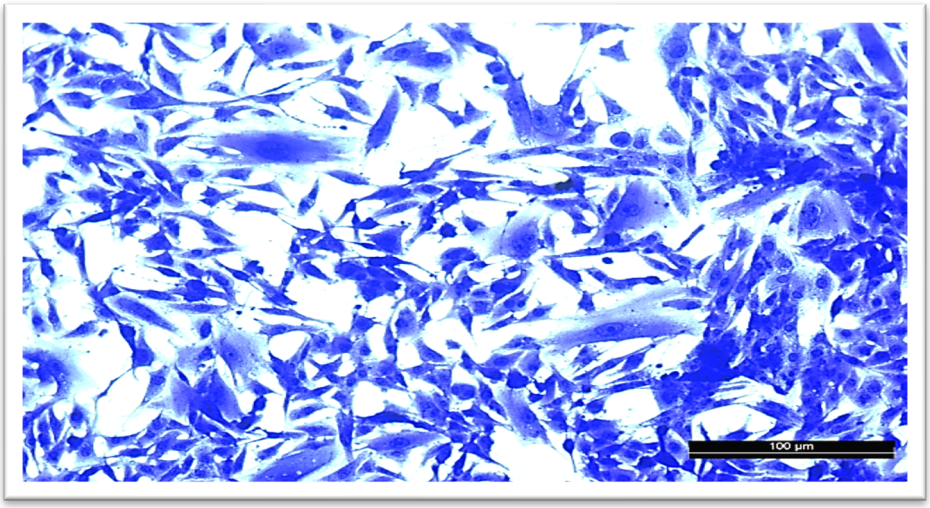

Fig. 3. HepG2 cell line treated with gold oxide nanoparticles and stained with crystal violet dye.

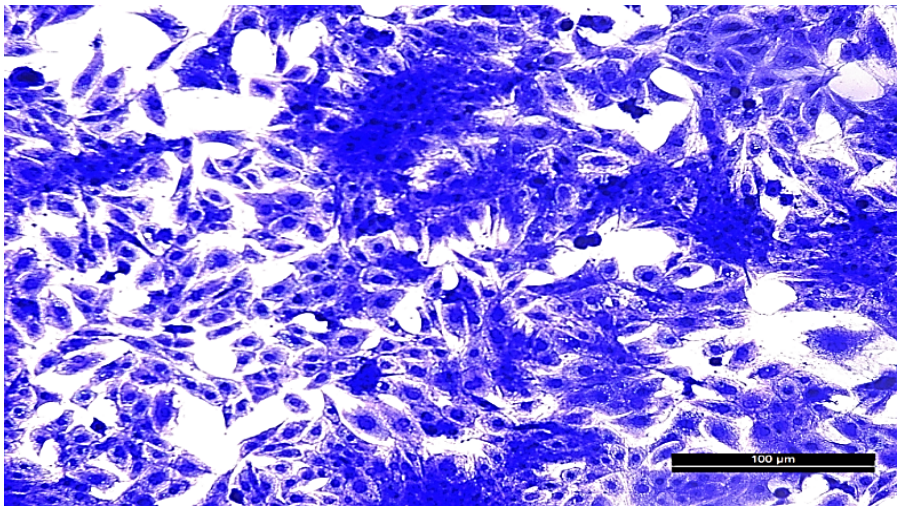

Fig. 4. MEF cell line with a concentration of 100 micrograms of gold oxide nanoparticles stained with crystal violet dye.

inhibition rate was 35.82%, and the inhibition rate increased to 78.20% at the highest concentration, which was 100 µg/ml. The results of the statistical analysis confirmed the presence of significant differences between the concentrations**.**

It was observed that MEF cells (normal cells of mouse embryos) showed fewer toxic results compared to cancer cells when exposed to gold oxide nanoparticles. At the concentration of 6.25 µg/mL, the inhibition rate of MEF cells was 5.12%, but the inhibition rate increased to 51.34% at the particle concentration of 100 µg/mL (Fig. 4).

The results indicate that the toxicological activity of gold oxide nanoparticles towards liver cells was higher compared to breast cancer cells and normal cells. The IC_{50} value for liver cancer cells was 12.22, while its value for breast cancer was 46.84 and for normal cells was 85.91. This indicates that the toxicity of gold oxide nanoparticles towards normal cells is very weak, that is, they affect these cells less than cancer cells (Fig. 5).

The cytotoxicity of gold oxide nanoparticles is effective in this study due to the small size of the nanoparticles, as multiple studies indicate that the effectiveness of gold nanoparticles in eliminating cancer cells increases when the particle size is smaller. The small size of nanoparticles is considered an important factor in causing cancer cell death [32, 33,34]. The results of the effectiveness of gold oxide nanoparticles against liver cancer in the current study are consistent with the study conducted [35], which used HepG2 cell lines. The study showed that gold nanoparticles possess good toxic activity against cancer cells and the concentration plays an important role in the anti-cancer activity. The study found that the best cancer growth inhibitory effect was achieved at a concentration of 100 µg, followed by 50 µg, 25 µg and 10 µg as the lowest cancer growth inhibitory concentration. Gold oxide nanoparticles were observed to have strong cytotoxic activity against cancer cell lines at varying concentrations. It is worth noting that the percentage of cytotoxicity on natural cells was very small, and these results were consistent with the study [36], which showed significant inhibition of gold nanoparticles against MCF-7 lines.

and PC-3 did not affect normal MCF-10 cells. Studies have shown that some other nanoparticles have selective cytotoxicity in some cancer cells. For example, in a study by [37], it was shown that iron oxide (Fe_3O_4) nanoparticles can kill cancer cells (A-549 and HepG2) without affecting healthy cells such as liver cells of rats and humans and lung fibroblast cells. IMR-90. The higher cytotoxicity of nanoparticles against cancer cells than normal

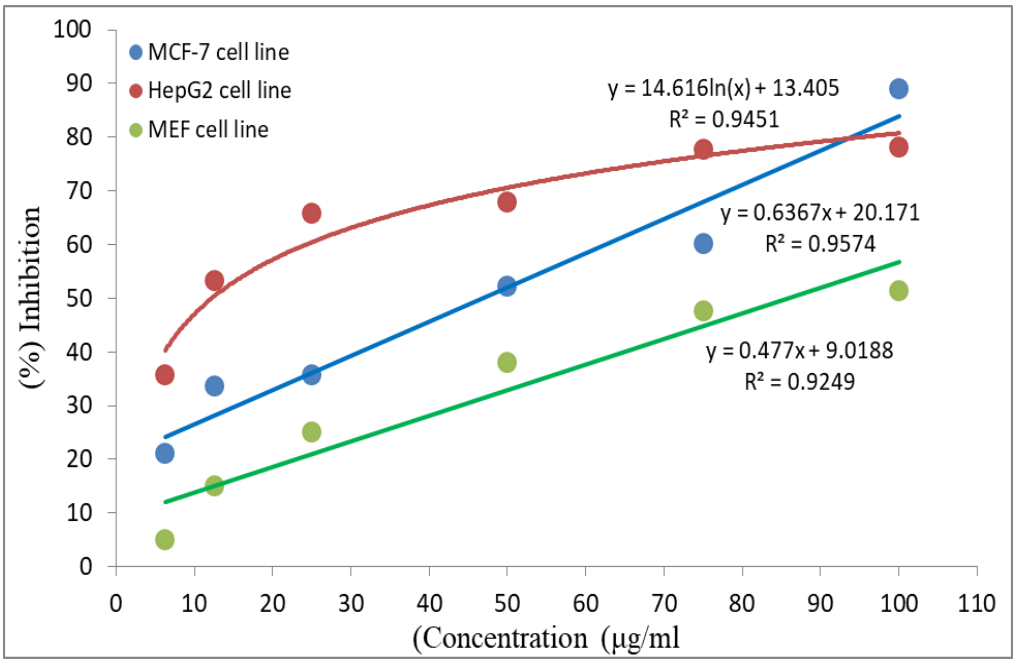

Fig. 5. IC_{50} curves of gold oxide nanoparticles for HepG2, MCF7, and MEF cells.

cells is explained by their higher uptake of these particles compared to normal cells. Cancer cells are believed to suffer from abnormal metabolism and rapid growth, making them more vulnerable to damage [38,39,47]. It was observed in this study that the liver cell line HepG2 was more resistant with an IC_{50} value than the breast cell line MCF7. This could be attributed to the expression level of Bcl-2, an anti-apoptotic protein [40]. After exposure to gold oxide nanoparticles, ROS are normally released from mitochondria. This can induce oxidative stress and reduce the expression of the Bcl-2 protein, which is also important for cytotoxic and apoptotic processes [41, 42]. However, increased expression of anti-apoptotic proteins such as the Bcl-2 family increases resistance to apoptosis and vice versa [43]. Another study indicated that the susceptibility of the A-549 cell line to isoorientin (a natural compound) was associated with decreased Bcl-2 protein production, which led to programmed cell death via a mitochondria-dependent mechanism [44]. A similar phenomenon was observed when A-549 cells were treated with copper oxide nanoparticles [45].

CONCLUSION

The study provides an environmentally friendly and cost-effective approach to the synthesis of gold oxide nanoparticles that are powerful against cancerous tumors and can be used as an alternative to commercially available chemotherapy treatments that have many negative effects on the human body. Gold oxide nanoparticles showed significant inhibition of the proliferation of HepG2 cells more than MCF-7 breast cells and MEF normal cells according to the IC50 value.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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