



Examination of the Toxicity of Gold Nanoparticles Manufactured by Green Methods from the Leaves of Lantana Camara Plant on Some Cell Lines

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Abstract

Gold nanoparticles were synthesized from the hot ethanolic extract not from the leaves of the enamel dendritic plant and their properties were studied by using the SEM scanning microscope to determine the surface shape and the structural size of the biosynthesized gold nanoparticles from the enamel dendritic plant. The results show that the nanoparticles ranged between 40-80 nm, the working force microscope was used as follows: Shapes, topography, roughness and bumps of gold nanoparticles, and the average roughness size of the nanoparticles 11.5 nm. Angle 51, angle 66 and angle 73 nm There is an X-ray diffraction database ICDD file. 220-220-311 UV-visible spectrometer was used to detect gold particles, which showed that the absorption peak of gold particles was at wavelength 400-480 nm and the highest absorption was at wavelength 480 nm. The particles have cytotoxicity against cancerous tumors through their effect on the cells of the liver cancer cell line (HEPG2) and compared it with the normal cells (WRL68) and the degree of its effect depends on the concentration. Dendritic enamel. The inhibition values were (37.35 and 3.59) and (400 and 25) µg/ml respectively for HEPG2 cell line. As for the normal cell line WRL68, it was as follows (26.81 and 4.2) for the same concentrations, and the IC50 concentration for cancer cells was (237.1) µg/ml and for the normal cell line (110.0) µg/ml when compared with the results of the alcoholic extract, which were on the cell line as follows (51.08) and 3.25 for the concentrations (400 and 25) µg/ml, respectively, and the inhibitory value of the extract on the normal cell line was (48.42 and 4.71) and for the same concentrations as above, it reached IC50 µg/ml for cancer cells 146.7 and for normal cells (150.0) µg/ml.

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Key Words: Toxicity, Nanoparticles, Green Methods, *Lantana Camara*.

DOI Number: 10.14704/nq.2022.20.10.NQ55255

NeuroQuantology 2022; 20(10): 2924-2931

Introduction

Medicinal plants have been for centuries a source of a large number of biologically active compounds that are widely used to treat various diseases, including cancer. It is also known that (*Lantana Camara*), which is found in Iraq from plants, contains many effective plant components such as (Terpenoids, flavonoids, alkaloids, etc.) against many diseases such as asthma, ulcers, cancer, etc. (Kumar et al., 2016).

Through what has been shown from the studies, dendritic enamel has been reported to have strong chemopreventive properties against various types of cancer and has been associated with high efficacy

rates when tested on animals (Sharma et al, 2007). However, dendritic enamel has been shown to cause acute hepatotoxicity at high doses. Recently, work has increased and the increasing acceptance of nanotechnology for its multiple use in most areas and that the importance of these materials is primarily due to their small size and availability in environmentally friendly materials (Zakir, 2014). These particles are derived from the Greek word nano, which means dwarf, and nano represents part of a billion the science that deals with the applications of using these materials is also called

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nanotechnology the active components in bio-molecule using nanoparticles have received great attention, and among the many metallic nanomaterials are gold and silver due to their unique physical and chemical properties.

For its applications such as anti-inflammatories, gene delivery, drug delivery, cancer treatment, the nanoparticles obtained by biological methods are free from toxic contamination by by-products and on the other hand, they are cost-effective and environmentally and biologically compatible with manufactured gold nanomaterials (Emily, 2010).

Materials and Working Methods

1. Collection of Plant Samples

Lantana camara leaves were collected from nurseries and home gardens in Baghdad during the month of October 2021. Confirmed by the National Botanical Herbarium / General Commission for Agricultural Research in Abu Ghraib near the College of Agriculture in the past.

2. Preparation of the Alcoholic Extract of Plants

The alcoholic extracts of the leaves of the traditional Mina shigeri plant were prepared by washing the fresh, healthy leaves with running water to remove the contaminants on their surface and soaking them for 30 minutes. After removing moisture from it and drying it well by dry air, it was cut with sterile scissors into small pieces and pieces, and mashed well, and the weight of the chopped plant was 10 g where the percentage of extraction was, and it was placed in a beaker of 250 ml capacity of 10 ml 100 distilled water, It was heated in a water bath at 45 degrees for about 30 minutes. Then the extracts were filtered using Watman No.1 filter paper and stored at 4°C for later use.

3. Biofabrication of Gold Nanoparticles

Gold nanoparticles were synthesized for the extract of shigeri enamel plant according to the method of (Lin et al., 2019). 10 ml of the aqueous extract of the shigeri enamel plant was mixed with 100 ml of 1 mmol gold chloride solution, and the mixture was heated on a magnetic vibrating thermal plate at a temperature of 45°C for 20 minutes, and the observation of the color change of the mixture was adopted as a clear evidence of the construction of gold nanoparticles, How was 100 ml of 1 mM gold nitrate solution prepared above for the purpose of

adopting it as a control in some measurements?

4. Detection and Characterization of Biosynthetic Gold Particles

The prepared samples were diagnosed compared to the alcoholic extract in the following ways:

4.1. Atomic force Microscopy (AFM)

Atomic force microscopy (Angstrom Advanced AA2000) was used to determine the size and diameter of gold nanoparticles as well as the surface shape and roughness of nanoparticles by placing a small drop of sample solution on an 11x cm glass slide and letting it dry at room temperature to be ready for testing (Qadar et al 2019).

4.2. Ultraviolet Visible (UV) Spectrophotometer

A sample was taken 48 hours after preparing the green nanoparticles, and it was examined by a UV spectrometer with a wavelength of 190-1100 nm. Deionized distilled water was used to check the optical properties of the product at room temperature (Bhrathidasan and Panneerselvam., 2012).

4.3. Scanning Electron Microscopy (SEM)

An electronic scanner of the French MIRA3 FE-SEM was used to determine the shape and size of the prepared samples (Vanmathi selvi and Sivakumar, 2012) by placing 5 µL of the prepared solutions on the electronic prepared holder made of gold and interlinked carbon carbon and leaving them to dry at room temperature and testing them using magnifying forces. different.

5. Cell Lines

In this study, the normal hepatocyte line WRL68 and the cancer cell line HEPG2, a non-radioactive colorimetric assay used to measure cell vitality in response to various cytotoxic stimuli, were used. MTT dye changed from yellow to purple formazan in living cells by NADH enzyme, and the cytotoxic effect of all extracts (aqueous, methanol, butanol, ethyl acetate, dichloromethane, diethyl ether) was determined on the liver line (vaquero et. al., 2007).



5.1. Methods of Sterilization

5.1.1. Wet Heat Sterilization

Liquid and solid culture media and the used solutions were sterilized with an auto clave at a temperature of 121 and a pressure of 121 and a pressure of 15 atmospheres for one minute (Sultana., 2007).

5.1.2. Dry Heat Sterilization

The inoculation needles were sterilized until they reached redness using direct gasoline flame, and the glassware was sterilized for two hours at 150 degrees Celsius in an electric oven (Ray and Ryan., 2004).

5.1.3. Sterilization by Chemical

The hood was sterilized with 70% ethyl alcohol and 30% chlorine/minor, respectively (sultana., 2007).

5.1.4. Sterilization by Filtration

The plant extracts and the heat-sensitive filtration prepared nano-solutions were sterilized using micro-filters with a 0.22 um osmotic diameter (Ray and Ryan., 2004).

5.2. Preparation of Solutions and Reagents Used in Tissue Culture

5.2.1. Mc Farland Standard Solution

Standard turbidity constant solution (Mc Farland Standard) To measure (calibrate) the density of the bacterial inoculum in the examination of sensitivity to antibiotics, a McFarland tube (Mc Farland NO. 0.5) was used in preparing the following solutions according to (NCCLs, 2003).

Solution A: Prepared by dissolving 1.175 g of aqueous barium chloride ($H_2O_2BaCl_2$) in 100 ml of sterile water.

Solution B: Prepared by adding 1 ml of concentrated sulfuric acid H_2O_4 to 100 ml of sterile distilled water, adding 0.5 ml of solution A to 99.5 of solution B with continuous shaking to obtain a (suspension), and kept in the dark with tight-fitting tubes to prevent evaporation. The density of the MacFarland scale 0.5 was verified at the wavelength of 625 nm, as it should give an absorbance between 0.1-0.08 and the equivalent of $1-2 \times 10^8$ colony forming units per ml.

5.2.2. Phosphate Buffered Saline (PBS)

This solution was prepared according to (Forbes et al., 2007), by dissolving 8.77 g of sodium chloride, 5.1 g of potassium dihydrogen phosphate (KH_2PO_4) and 10 g of sodium monohydrogen phosphate ($Na_2HPO_4 \cdot 7H_2O$) in 500 ml of distilled water. Adjust the pH at 7.2 and fill the volume to a liter with distilled water. Then sterilize and store at $4^\circ C$ in the refrigerator.

6.1. MTT Test to Check Cell Viability After Treatment with Zinc Oxide Particles

This method was done according to (Rashid H. et al 2017).

1. HEPG2 cancer cells were prepared, then the cell suspension was placed in a flat-bottomed 96-hole plate and incubated in a 5% CO2 incubator at $37^\circ C$ for 24 hours.
2. After incubation, 100 microliters were added to each hole of the cell suspension.
3. The concentrations prepared from the extract of the leaves of the enamel shigeri plant were added (25, 50, 400, 200, 100) microliters at a rate of (3) holes for each concentration.
4. The plate was incubated for 24 hours at $37^\circ C$.
5. 10 μl of MTT solution was added to each pit at a concentration of 0.45 mg/ml.
6. The plate was incubated for 4 hours at $37^\circ C$.
7. 100 microliters of solubilization solution (DMSO) were applied to each pit in order to dissolve the Formazan crystals.
8. The absorbance of the sample was read at a wavelength of 570 nm using an ELISA device to a statistical analysis to determine the concentration of nanoparticles and the alcoholic extract IC50 necessary for a decrease in the growth capacity of each cell line according to the equation.

$$\text{Viability \%} = \frac{\text{optical density of sample}}{\text{optical density of control}} \times 100$$

In the same way, the toxicity of gold nanoparticles was examined on the normal WRL68 cell line.



Results and Discussion

Morphological properties of aqueous extracts of dendritic enamel leaves and biosynthesized gold nanoparticles.

The results showed that the alcoholic extract of the leaves of the dendritic enamel plant was prepared, that the final appearance of the extract of this plant was dark brown, while the biosynthesized gold nanoparticles from the enamel plant appeared in violet color. Reduction process of gold chloride particles to gold nanoparticles as shown in Figure (1) This result is consistent with the study conducted by (Dan et al., 2015) that the color changes are a visual evidence for the initial detection of the occurrence of the bioreduction process and that reliance on the color changes as a visual guide in the initial detection of the extracellular biosynthesis of gold nanoparticles and that the color changes come In full agreement with the results of previous research (AL-Othaman et al., 2017).

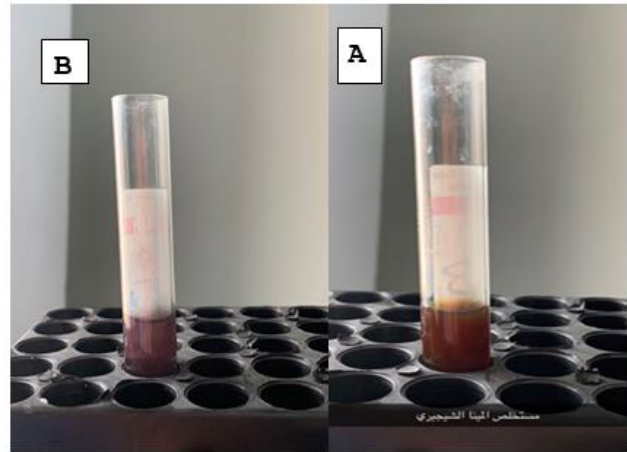


Figure 1. A shows the color of the alcoholic extract; B - color when forming nanoparticles

Detection and Characterization of Gold Nanoparticles for p Lant Extract in Comparison with Alcoholic Extract:

Diagnosis of Zinc Oxide Nanoparticles and Aqueous Extract Using Atomic Force Microscopy (AFM)

When conducting atomic force microscopy, Figure (4-3) shows that the size of the biosynthetic gold nanoparticles is 18 nanometers, with a swallowing roughness of 61.56, and here the difference is clear from the crude alcoholic extract, which has a size of 11.55 particles of the extract and a roughness rate of 27.23. (Zhang et al, 2017) This is consistent with the study of. The average diameter of nanoparticles ranges between 1-18 nanometers.

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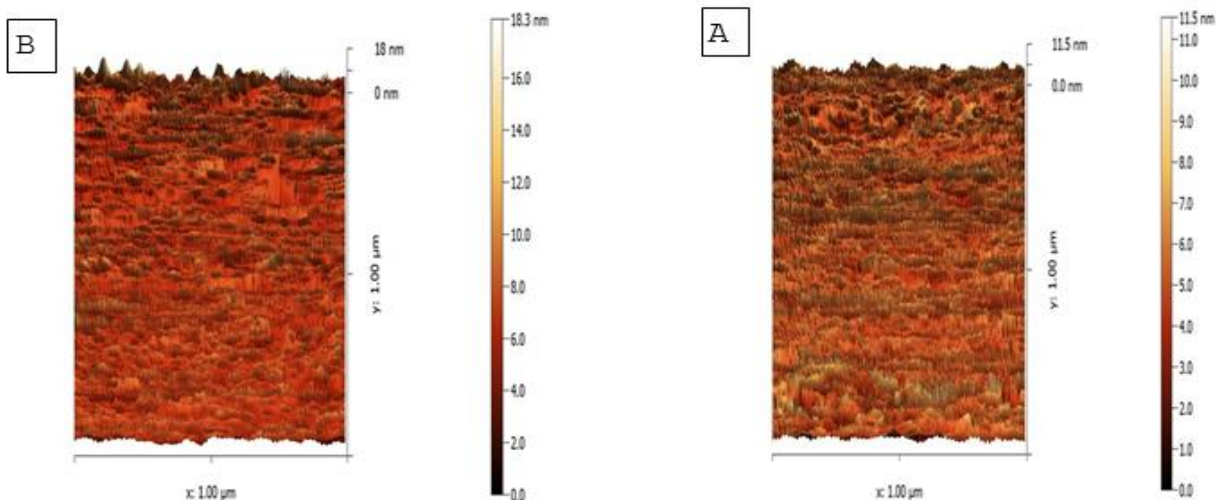


Figure (2a). Diagnosis of synthetic nanoparticles using an atomic force test device, where A: the alcoholic extract B: the biosynthetic nanoparticles



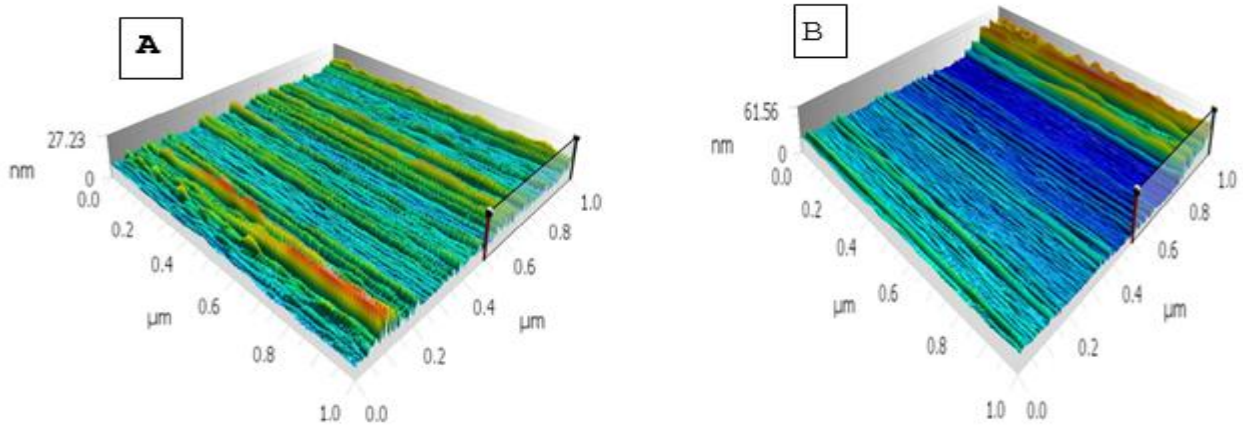


Figure 2b. Shows the Roughness Rate of Plant Alcoholic Extract and Gold Nanoparticle

A - For Plant Extract B-Gold Nanoparticles

Diagnosis of Nanoparticles and Aqueous Extract Using UV Spectroscopy

The results of the characterization using a spectrophotometer to detect gold nanoparticles for the extract of Shigeri enamel showed that the highest absorption peak was at the wavelength ($\max\lambda$) 480 nm because the absorption peaks that appear at the wavelength ranging between 400-480 nm is a characteristic of the diagnostic properties of gold nanoparticles. Gold nanoparticles due to surface plasmon absorption (SPR), and this

phenomenon occurs due to dipole oscillation, which is formed when the electromagnetic field located within the visible range is combined with the collective oscillation of conduction electrons, and this phenomenon often occurs on metal surfaces (gold and silver) When the light beam is directed, which strikes the metal surface at a certain angle, and this depends on the thickness of the molecular layer on the metal surface (Jaluse, 2017), and therefore the size of the formed particles can be counted within the limits and sizes of nanomaterials. (Palem et al, 2018).

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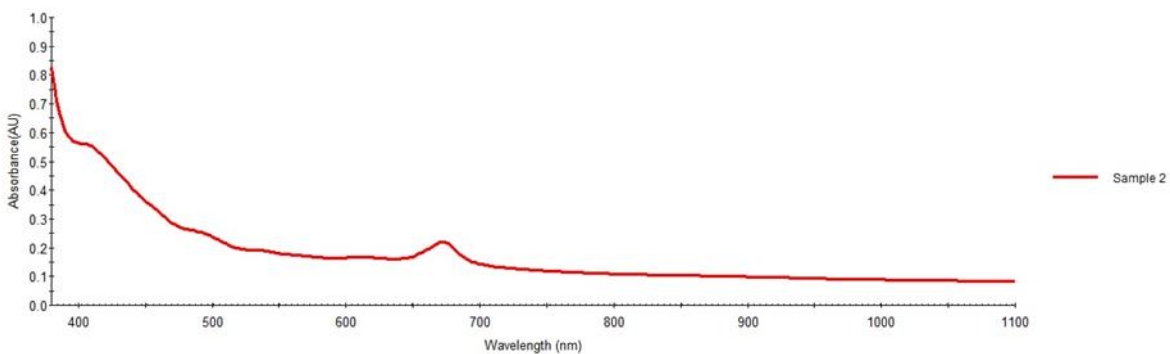


Figure 3. Characterization of Gold Nanoparticles and Aqueous Extract Using a UV Absorber

Analysis of Nanoparticle Images and Aqueous Extract Using Scanning Electron Microscopy (SEM)

By using a scanning electron microscope, a characterization was done to determine the structure and structure of each of the gold

nanoparticles and the plant extract, and it was noted that they are not lumpy and almost spherical in shape. It agrees with the results of multiple researches of gold nanoparticles (Mock et al, 2002).



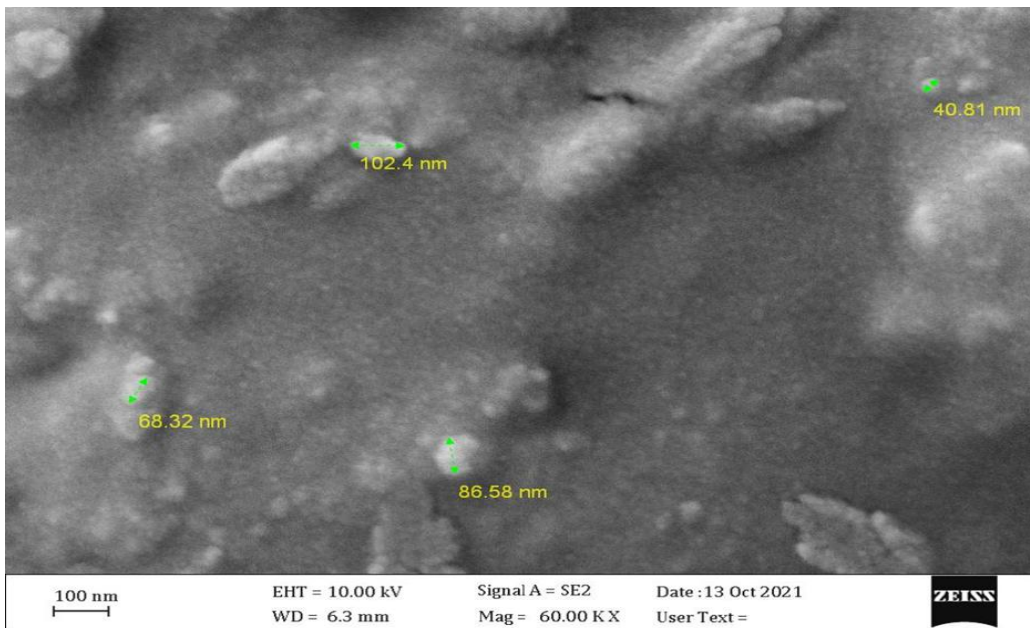


Figure 4. Image showing the nanoscale sizes of gold nanoparticles by SEM

MTT Cytotoxicity Test

At the present time, the importance of finding alternative materials and compounds for chemical drugs that are used as treatments, including cancer diseases, has become very important, and after testing the cytotoxicity of MTT for the purpose of the effect of each of tetragonal gold chloride and alcoholic extract from the plant enamel dendritic and combining the two together on the liver cancer HepG2 line, thus using Several concentrations of each compound on these cells, and the results showed that gold nanoparticles had an effect on cancer cells, as the vitality of cancer cells at a concentration of 400 micrograms reached 48.92, and the inhibition value for normal cells at the same concentration was 27.35, and the inhibitory concentration for half of the experimental cells was Figure (5). For cancer cells 111.0 µg/ml and for normal cells 273.1 µg/ml. It is worth noting that the lower the IC50 value, the greater the toxicity of the substance and its ability to inhibit cells.

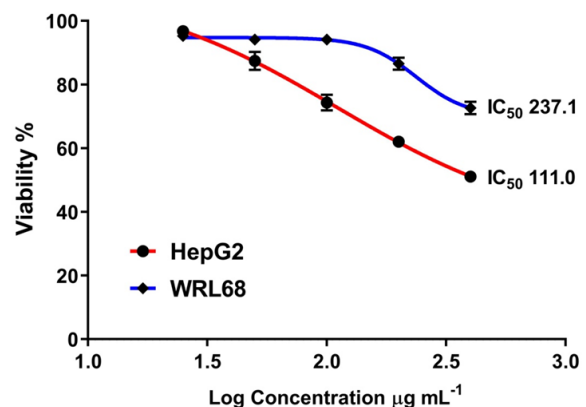


Figure 5. Curve showing the IC50 values of HEPG2 cancer cell line and WRL68 normal cell line when treated with gold nanoparticles

As shown in Table (2), the use of the crude alcoholic extract with different concentrations on cancerous and normal liver cells, as at the concentration of 400 µg / ml, the inhibitory value of the cancer cells was 37.35 µg / ml, and the inhibitory value of the normal liver at the same concentration was 26.81 µg / ml. The inhibition at concentration 25 for cancer cells was 5.94 µg/ml, and for normal cells for the same concentration was 4.2 µg/ml, and the IC50 on cancer cells was 150.0 µg/ml and for normal hepatocytes was 146.7 µg/ml.

Table 1. Represents the inhibitory value of cancerous liver cells and normal liver cells

ug/ml	Cancer liver (HePG2) %	(WRL68) Normal liver %
400	51.08	48.92
200	37.92	13.46
100	25.66	5.86
50	12.54	5.86
25	3.24	4.71



Table 2. Represents the vitality of cancerous liver cells and normal liver cells

ug/ml	Cancer liver (HEPG2) %	Normal liver (WRL68) %
400	37.35	26.81
200	27.62	19.79
100	15.93	13.58
50	5.94	5.05
25	3.59	4.2

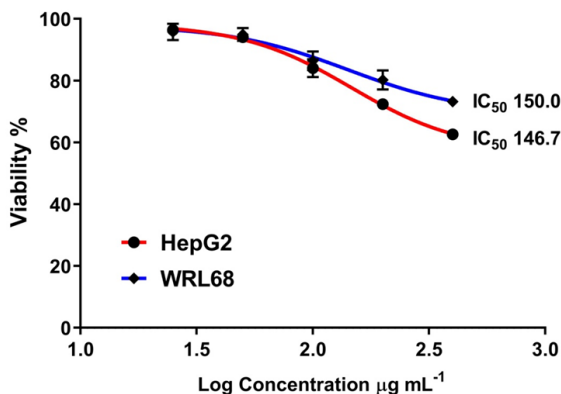


Figure 6. Shows the effect of the raw material on the liver cancer cell line HEPG2 and the normal cell line WRL68

Through these results, it was demonstrated that the prepared gold nanoparticles had a high activity on liver cancer cells and less toxicity on normal liver cells compared to the crude alcoholic extract. On the amount of concentrations used for the active compounds against liver cancer cells, as these nanoparticles are characterized by their small size, biocompatibility and accumulation in tumor sites. The other and most important reason is that it has the ability to bind with many proteins and drugs that specifically target active cancer cells, and that the toxicity of gold particles on cells is only the result of a chemical-physical interaction of gold atoms with the functional group of intracellular proteins. With nitrogenous bases and phosphate groups in the synthesis of DNA (Peng et al., 2019), these results enable us to arrive at that gold nanoparticles are used to inhibit cancer cells through their strong effectiveness as they are molecules that can be formed in easy, fast and environmentally friendly ways.

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