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Original Article

Investigation of Antioxidant and Cytotoxicity Effects of Silver Nanoparticles Produced by Biosynthesis Using *Lactobacillus gasseri*

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Abstract

The biosynthesis of silver nanoparticles (AgNPs) is a new approach in nanotechnology which was optimistically implemented in medicine, food control, and pharmacology. The present study aimed to investigate the antioxidant and cytotoxicity effects of AgNPs produced by *Lactobacillus gasseri* filtrate. Also, changing color from yellow to brown confirmed the production of AgNPs. AgNPs were characterized using ultraviolet-visible spectroscopy, FE-SEM, and Fourier Transform Infrared Spectroscopy (FTIR). The antioxidant activity of AgNPs was tested using the DPPH assay. The scavenging test for DPPH showed 19.3%, 32.6%, 47.6%, 72%, 85.3% at concentrations (6.25, 12.5, 25, 52, 100) µg/ml, respectively, which proved that the scavenging percentage increased with increasing concentration. The effect of AgNPs on the chromosomal pattern was also studied. The results of the experiment of AgNPs against SK-GT-4 cancer cells showed the toxic activity of the used particles against the strains of these human esophageal cancer cells and failed to affect normal cells.

Keywords: Cancer Cells, Chromosomal Aberrations, Nanoparticles

1. Introduction

The term "probiotic" was first used in 1965 by Lilly and Stillwell to describe substances secreted by one organism which stimulate the growth of another. Probiotics encourage the multiplication of other bacteria, often known as the normal flora, or types of beneficial bacteria that are cultivated in the laboratory and kept alive in special packages to reach millions or billions of bacterial cells in a single dose. Probiotics were discovered in 1953 and have been particularly effective in treating gastrointestinal diseases (1). Probiotics are completely absorbing foods and preventing inflammatory bowel diseases, colon cancer, and lactose intolerance, especially in infants, reducing flatulence, treating constipation and colic, as well as nutritional deficiencies by producing several vitamins in the gastrointestinal tract, reducing fungal infections in the vagina, especially those caused by *C. albicans*, which endanger the health of pregnant women. *Lactobacillus* is one of the most important species of bacteria used in manufacturing probiotics (2).

Lactobacillus spp. are on-spore-forming, rod-shaped, facultative anaerobic, catalase-negative, (Gram +ve) which grow better under microaerophilic conditions. Their Gram stain morphology can vary, including short, plump rods, long, thin rods, in chains or palisades. Also, their colonial morphology ranges from small to larger gray colonies with alpha hemolysis on blood agar. *Lactobacillus* can also be grown on other media, such as MRS (Man, Rogosa, and Sharpe) agar, where they appear as white, mucoid colonies. *Lactobacillus* consists of about 170 species and 17

subspecies, all of which have been formally described and have a solid nomenclatural standing. They are naturally found in the gastrointestinal system and vaginal canal in humans, however can also be opportunistic pathogens (3).

Silver metal has been known since 4000 B.C. which was used in many medical uses, even before it was realized that microorganisms are the main cause of infection (4). Also, producing nanoscale silver became possible with the emergence of nanotechnology (5, 6). Silver nanoparticles have been used against reactive oxygen species (ROS), which gave excellent results. Antioxidants are known to have many benefits in scavenging free radicals and eliminating many cardiovascular and cancers of the body (7). Silver nanoparticles have also been used in various types of cancer lines, and it has been observed that tumor progression and the disease are inhibited by them without causing toxicity to normal cells (8). Silver nanoparticles can be synthesized using traditional or unconventional methods, with two different approaches of top-down and down-top. Many traditional methods are used to obtain silver nanoparticles, such as chemical/photochemical reactions, thermal decomposition of various silver compounds, electrochemical, radiation, and microwave-assisted methods (9). Unconventional methods of creating these particles depend on using microorganisms such as bacteria, fungi, marine algae, and yeasts or various extracts of alcoholic or aqueous plants, as they are considered as reducing or inhibitory agents (10). Green synthesis methods are the best due to their many advantages in preparing silver nanoparticles: they are low cost, environment friendly, and do not require high pressure, energy, or the use of chemical reagents (11). The present study aims to perform the biosynthesis of AgNPs using *L. gasseri* bacterial filtrate with silver nitrate solution and to investigate the cytotoxicity of silver nanoparticles on cancer cells.

2. Material and Methods

2.1. Collection of Bacterial Isolates

Twenty isolates for *Lactobacillus* sp. were diagnosed with a VITEK device from the Microbiology Laboratory at Al-Olwiya Teaching Hospital for Children in Baghdad. The isolates then were planted on the surface of the slanted bed medium formed from De Man, Rogosa, Sharpe agar (MRS) agar, and then incubated and kept at 4°C until use.

2.2. Morphological and Microscopic Examination

Isolates were grown on MRS agar to determine the color and size of colonies and then stained with Gram stain.

2.3. Maintenance of Cell Cultures

SK-GT-4 cells were maintained in RPMI-1640 supplemented with 10% Fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were passaged using Trypsin-EDTA reseeded at 80% confluence twice a week and incubated at 37 °C (12).

2.4. Biosynthesis and Purification of Silver Nanoparticles Using *L. gasseri* Bacteria

The bacteria were seeded in an MRS agar medium for 48 hours, and then the growth medium was discarded by a 4000 rpm centrifuge for 10 minutes. The precipitate was removed and the filtrate sterilized with filters of 0.22 µm pore and preserved until use (13). Silver nanoparticles were synthesized using a filtrate of *L.gasseri* bacteria; 10 ml of culture filtrate was mixed with 90 ml of 5 mM silver nitrate solution and incubated at room temperature for 48 hours. The synthesized silver nanoparticles were discovered in the reaction mixture by detecting the color alteration of the medium from pale yellow to brown as well as observed optical density (13). The aqueous solution containing AgNPs was placed in test tubes for purifying AgNPs and then centrifuged at 10,000 rpm, for 10 minutes. One minute for 10 minutes and the process was repeated 3 times until the filtrate became colorless, and the concentrated precipitate containing silver nanoparticles was placed in an hour bottle and dried in an electric oven at 50° C (the thermal drying method) so that excess water was removed and then the precipitate was collected after drying and preserving until use (14).

2.5. Characterization of Silver Nanoparticles

Take 1 ml of a solution containing silver nanoparticles and add 9 ml of deionized water. The aqueous solution was then measured to reveal the nanoparticles formed at a wavelength ranging from 300 to 600 nm. The highest absorption (λ max) appeared after the wavelength of 400 nm, indicating the formation of silver nanoparticles (15).

FTIR was used to characterize nanoparticles. The bio-produced silver solution was mixed with potassium bromide at a ratio of 1: 100 and then tested using FTIR Infrared Spectrometer within the range of 4000-400 cm^{-1} (15).

A scanning electron microscope (FE-SEM) was used to determine the particles size and morphology of AgNPs. The sample was dispersed on a smooth surface of carbon base and then coated with a thin layer of gold and subjected to examination. EDX equipped included with FE-SEM was used to determine the chemical elements of AgNPs.

2.6. Antioxidant Activity of AgNPs

Antioxidant activity of AgNPs was measured using stable 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radicals with minor adjustments according to (16, 17). Silver nanoparticles were used in concentrations (6.25µg/ml, 12.5 µg/ml, 25 µg/ml, 25 µg/ml, 100 µg/ml) to investigate the scavenging activity. The samples were mixed with 450 µl of DPPH solution and then the volume of the mixture was completed to 1 ml using absolute ethanol. Ascorbic acid was used as a positive control at a concentration of 100 µg/ml. The samples and control are kept in dark at room temperature for 30 minutes. The absorbance was measured at 517 nm. Scavenging activity was measured according to the equation formula:

$$Scavengingi\% = \frac{Absorbance\ ioficontrol - Abosrbanceiofisample}{Absorbance\ ioficontrol} \text{xi} 100\%$$

2.7. Cytotoxicity Assays of AgNPs

MTT assay was performed using 96-well plates to determine the cytotoxic effect of AgNPs (18). Cell lines were seeded at 1 × 10^4 cells/well. After 24 h the attached monolayer was obtained. Furthermore, the cells were treated with the tested compounds at different concentrations. Cell viability was measured after 72 h of treatment by removing the medium, adding 28 µL of 2 mg/mL solution of MTT, and incubating the cells for 2.5 h at 37 °C. After removing the MTT solution, the crystals remaining in the wells were solubilized by adding 130 µL of DMSO (Dimethyl Sulphoxide) and then incubating at 37 ° C for 15 minutes by shaking (19). The absorbency was determined on a microplate reader at 492 nm; the assay was performed in triplicate. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation (20).

Inhibition rate = A- B/A*100; where A is the optical density of control, and B is that of the samples (21).

The cell was seeded into 24-well micro-titration plates at a density of 1×10^5 cells mL^{-1} and incubated for 24 h at 37 °C to visualize the shape of the cells under an inverted microscope. Then, cells were exposed to AgNPs at IC_{50} for 24 h after which the plates were stained with crystal violet and incubated at 37 °C for 10–15 min (22). The stain was washed off gently with tap water until the dye was completely removed. The cells were observed under an inverted microscope at 40× magnification and the images were captured with a digital camera attached to the microscope (23).

2.8. Study Effect of Silver Nanoparticles on the Chromosomal Pattern

Transplantation was performed to obtain chromosomes according to the method mentioned in (24) by adding 0.5 ml of peripheral blood to 4.5 ml of complete culture medium (RPMI-1640) prepared with 10 µg/ml of phytohemagglutinin (PHA) stimulator and two concentrations. Then two different concentrations of silver nanoparticles were biologically prepared using *L.gasseri* filtrate (0.0, 27, 54) µg/ml, and a positive control tube containing 0.65 µg/ml of methotrexate (MTX) was counted as a positive control. The tubes were incubated tilted at 37 °C for 70 hours, and colchicine solution was added at a final concentration of 10 µg/ml. The tubes were then returned to the incubator

at 37 °C for 2 hours, after which the implant was centrifuged at a speed of 2000 rpm for 10 minutes and the filtrate was neglected. The precipitate was suspended with 5 ml of hypotonic calcium chloride solution, which was gradually added by continuous shaking. Then the tubes were returned to the incubator for another 45 minutes to detonate blood cells in the (KCL) solution, and swelling of lymphocytes preparesto create its chromosomes. Then the contents of the tubes were centrifuged at 2000 rpm for 10 minutes to remove the filtrate and suspend the precipitate with a cold fixative solution which was immediately prepared by mixing three parts of absolute methanol with one part of glacial acetic acid. This solution is in the form of drops on the wall of the tube with continuous shaking to reach a volume of approximately 5 ml, after which the centrifugation process is carried out at a speed of 2000 rpm. This process was repeated several times to decolorize the solution, then the precipitated cells were suspended with (1.5 - 1 ml) of the fixative solution, mixed by a clean, dry Pasteur pipette, ready for installation on cool, wet slides. A certain size of the prepared cells was taken from above and 7 spaced drops were poured on the glass slide at a height of about 30 cm to obtain a good spread of chromosomes for easy observation of chromosomal changes.

The slides were completely air-dried in the previous paragraph, and they were stained with Giemsa stain for two minutes, then washed with distilled water, during

which Blast Index (BI) and Mitotic Index (MI) were calculated according to the following equations.

$$MI = \frac{Mitotic\ cells}{1000\ cell} \times 100\%$$

$$BI = \frac{stimulated\ cells}{1000 cell} \times 100\%$$

Chromosomal aberrations= summation of total abnormalities in 25 mitotic cells.

2.9. Statistical Analysis

The obtained data were statically analyzed using an unpaired t-test with GraphPad Prism 6. The values were presented as the mean ± SD of triplicate measurements (25).

3. Results

3.1. Morphological and microscopic examination

L.gasseri bacteria grew on De Man, Rogosa, Sharpe agar (MRS) by streaking method and incubated for 24 hours at 37 ° C. and the results are as shown in (Figure 1A). Microscopic examination was performed using a Gram stain and the cells were examined with a light microscope to identify the shape, color, and size of the cells. The results are shown in (Figure 1B).

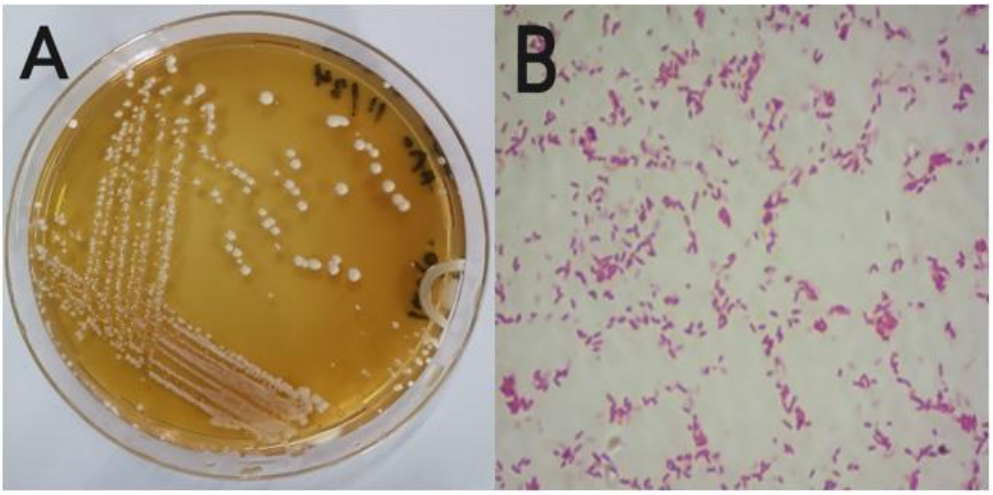

Figure 1. Morphological and microscopic examination of **A**= Growth of *L. gasseri* bacteria on MRS agar B= *L. gasseri* bacteria with gram stain

3.2. Biosynthesis of Silver Nanoparticle

The results of the biosynthesis process using *L.gasseri* bacteria filtrate with $AgNO_3$ solution at a concentration of 5 mmol, at pH of 6.5 and room temperature of 37°C for 48 hours were shown on the synthesis of AgNPs, and the change in color of the mixture to brown was evidence of a positive synthesis process (Figure 2A and Figure 2B).

3.3. Characterization of AgNPs Using UV-Visible Spectrophotometer

The characterization of the silver nanoparticles created using a UV visible spectrophotometer showed the highest absorbance (λmax) at the wavelength of 424 nm (Figure 3).

3.4. Characterization of AgNPs Using Fourier Transform Infrared (FTIR) Spectrometer

The samples were examined by FTIR device. The spectral scanning of the samples was conducted within the range of (400-4000) cm^{-1}. The spectral absorption results of silver nanoparticles showed the presence of bands at the range of (3368.79), (2958.69), (1632.3), (1399.38) and (1060.21) on the presence of the O–H, C–H, C=O, C–H and C–N bonds, respectively, which is consistent with the results of (26, 27) as shown in (Figure 4).

FTIR assay is one of the precise laboratory tests used to identify the chemical elements in the compounds, in which the identity of the chemical compounds is determined based on how chemical bonds in the compounds absorb infrared radiation, as each compound has its absorption (28).

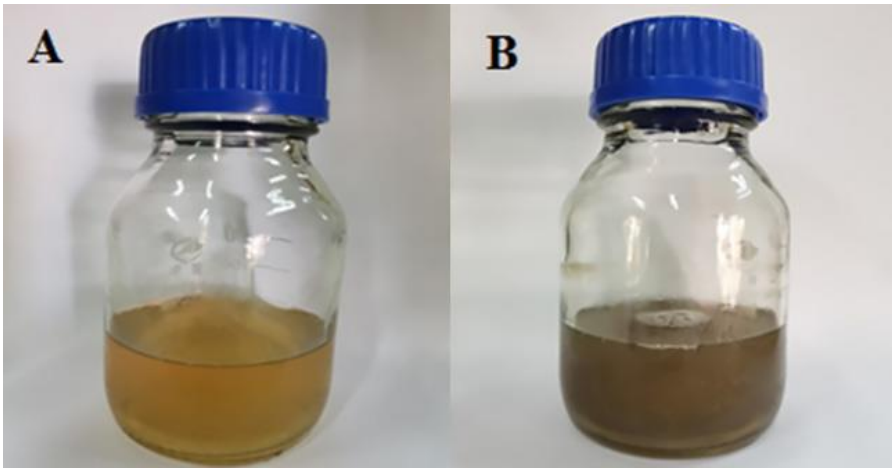

Figure 2. Stages of the synthesis of silver nanoparticles A=at zero time, B=after 48 hours

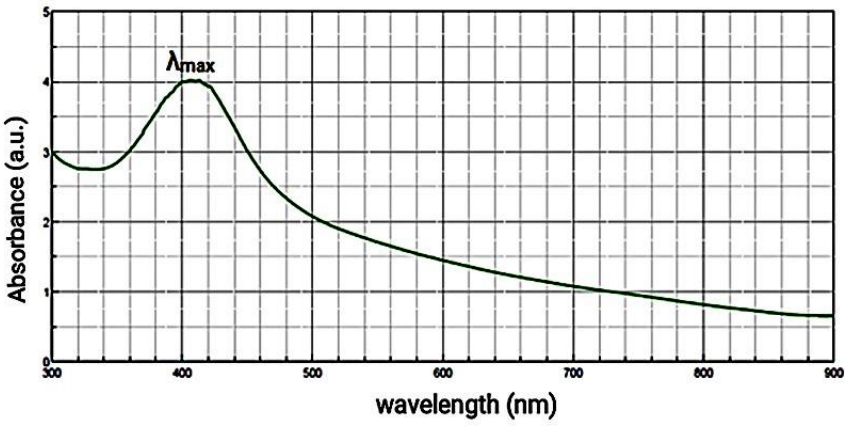

Figure 3. Characterization of silver nanoparticles using a UV visible spectrophotometer

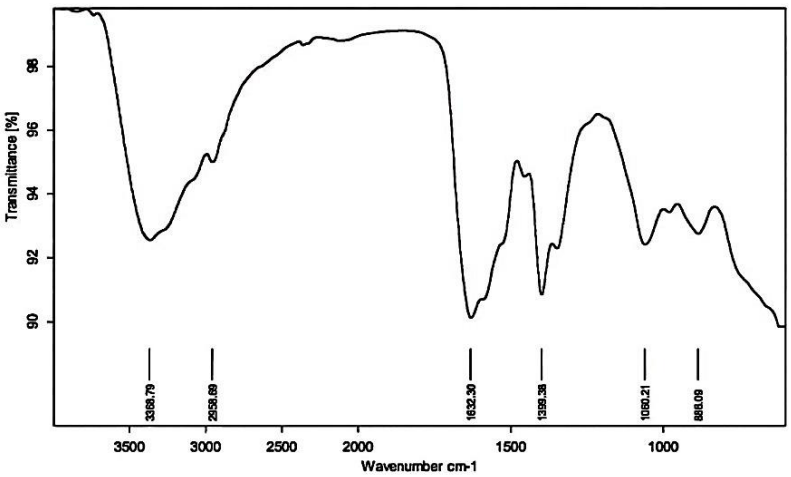

Figure 4. Fourier Transform Infrared (FTIR) spectrometer for silver nanoparticles

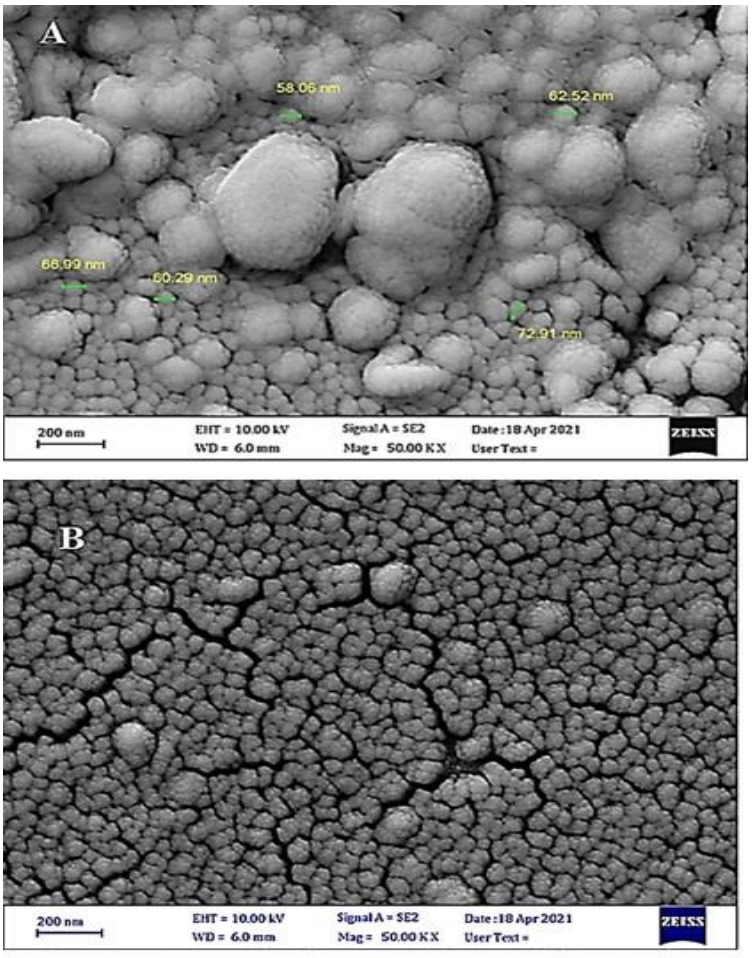

Figure 5. FE-SEM images for AgNPs A) Average size of AgNPs. B) Top view of AgNPs

3.5. Characterization of AgNPs Using FE-SEM Analysis

The FE-SEM analysis was conducted to evaluate the size, surface morphology, and uniformity of nanoparticles. It is a technique used for obtaining qualitative and quantitative information and provides further approaches to the morphology and size of the nanoparticles (29). Most AgNPs displayed a more obvious structural arrangement, having spherical and smooth surfaces with an observation of particles agglomeration and aggregation with a size range between (58.06 to72.91) nm, as shown in figure 5A and figure 5B.

EDX results showed that silver atoms contain 71.3 % of the total sample components. Also, a small percentage of Cl, O, C, S, Si noted that the peak of Au is present due to coating sample with gold in FE-SEM, as shown in figure 6.

3.6. Antioxidant Activity Using DPPH Radicals Scavenging Assay

DPPH is a free radical that is stable at room temperature, produces a dark violet color when dissolved in organic solvents, and is responsible for absorbance at the wavelength of 517 nm. DPPH reduces and turns yellow when silver nanoparticles are present due to the presence of a phenolic hydroxyl group (30). Figure 7 shows the antioxidant activity of silver nanoparticles using different concentrations, and the results of table 1 showed that the antioxidant activity of silver nanoparticles at a concentration of 100 µg/ml and 6.25 µg/ml were 85.3% and 19.3%, respectively.

3.7. Cytotoxicity Effect of Silver Nanoparticles against Cancer Cell Line

The cytotoxic effect of AgNPs against SK-GT-4 cells was studied. The antitumor activity of the AgNPs was tested by studying their ability to inhibit the proliferation of tested cells. The results of this study showed highly significant cytotoxic activity of AgNPs against the cancer cell line, however, not normal cell lines as shown in figure 8, figure 9 and figure 10. The results suggest that AgNPs suppress the growth of cell lines with a concentration-dependent effect (Table 2).

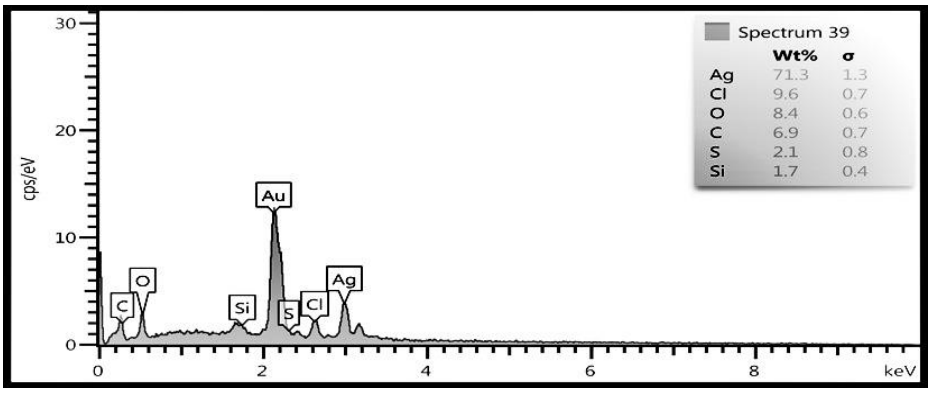

Figure 6. EDX spectrum for AgNPs

Table 1. Antioxidant activity using DPPH assay.

Silver nanoparticles	
Concentration µg/ml	**Antioxidant Activity %**
6.25	19.3
12.5	32.6
25	47.6
50	72
100	85.3

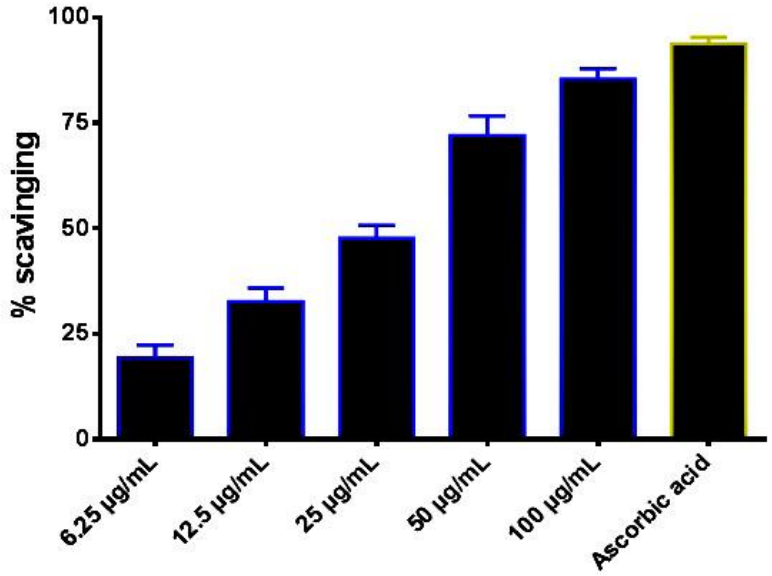

Figure 7. Antioxidant activity of AgNPs using DPPH assay, Ascorbic acid represents the positive control

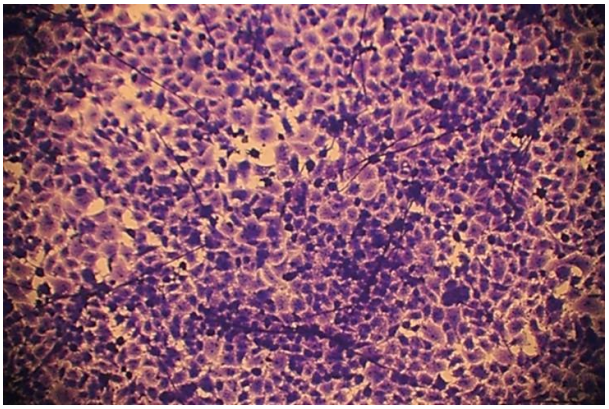

Figure 8. Control untreated SK-GT-4 cells

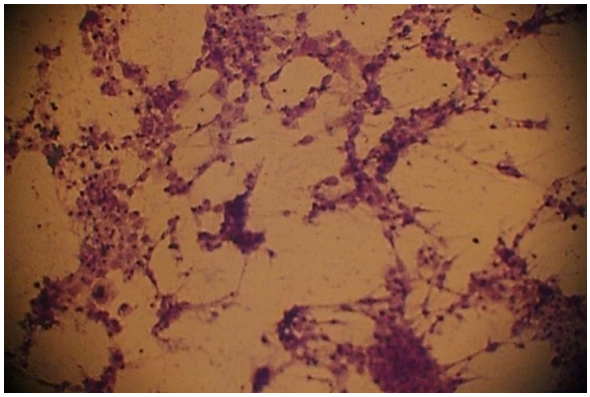

Figure 9. Morphological changes in SK-GT-4 cell after treatment with AgNPs

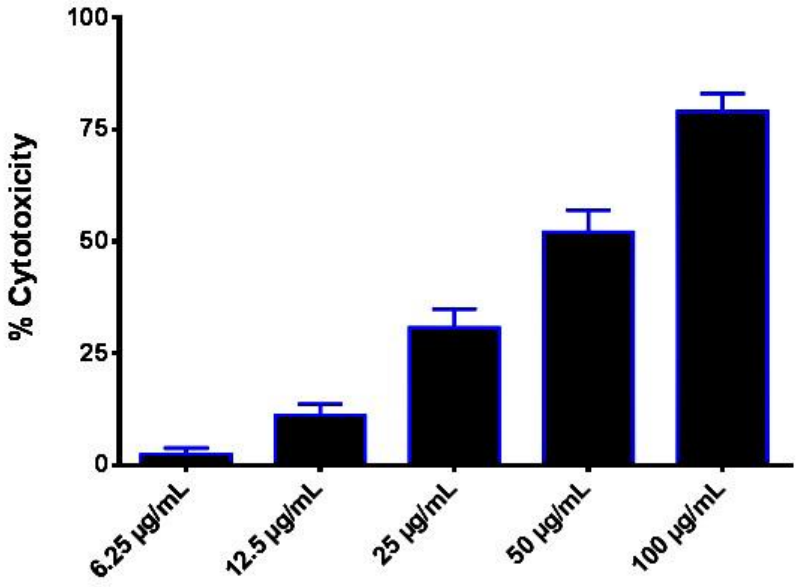

Figure 10. Cytotoxic effect of AgNPs in SK-GT-4 cells. IC_{50}=26.92 µg/ml

Table 2. The toxic activity of AgNPs against SK-GT-4 cells at different concentrations

Concentration µg/ml	Mean±SEM
6.25	2.33 ±0.882
12.5	11 ±1.527
25	30.66 ±2.404
50	52 ±2.886
100	79 ±2.309

Standard Error Mean = SEM

Table 3. Chromosomal aberrations induced in peripheral blood lymphocytes (PBLs) treated with AgNPs

Silver nanoparticles			
Concentration µg/mL	**BI**	**MI**	**TCA**
0.0	66.22	1.25	0.24
27	62.12	1.12	0.29
54	60.51	0.92	0.31
MTX0.65	16.55	0.11	0.52

BI=Blast Index, MI= Mitotic Index, TCA= Total Chromosomal Aberrations

3.8. Effect of Silver Nanoparticles on the Chromosomal Pattern

Table 3 shows that the effect of chromosomal aberrations (MI) is inversely proportional to the increase in AgNPs concentrations, which leads to chromosomal abnormalities through the appearance of diploid and ring chromosomes after treatment with concentrations of silver nanoparticles. The decrease in the indicators of MI and BI occurred as a result of cell death or stopping at interphase and the effect of silver nanoparticles on its division.

4. Discussion

In the biosynthesis of silver nanoparticles, discoloration of the solution explains the formation of the silver nanoparticles by existing enzymes, such as the nitrate reductase, which the bacteria secrete from the cell and reduces silver nitrate to nanoparticles (31).

The characterization of AgNPs using a UV visible spectrophotometer is one of the most important tests used to verify the formation of silver nanoparticles which depends on the extent of the optical absorption between wavelengths (300-900) nm to investigate the size of the nanoparticles, which ranges between (2-100) nm, as metals of nanoscale have free electrons which give (SPR) depending on their size, which results from the vibrations of the metal's electrons compared to light waves. By showing (SPR) after the wavelength of 400 nanometers in the process of synthesizing silver nanoparticles using *L.gasser*i filtrate, the results were in line with (32).

The antioxidant activity of silver nanoparticles revealed that this effect increases with increasing concentration but remains lower compared to ascorbic acid, this agrees with (33). Plant-mediated AgNPs production has also been investigated, and researchers have shown that antioxidant activity gradually increases by increasing therapeutic doses (34). Antioxidants are beneficial in the treatment of illnesses such as neurological disorders, cancers, and AIDS due to their scavenging capability (35).

The unique ability of silver nanoparticles to kill cancer cells has been proven by targeting the structure and function of mitochondria, in which silver accumulates and affects the respiratory chain of the cell, causing its programmed death (Apoptosis) (36).

Some studies have shown that small silver nanoparticles enter the nucleus, affecting its chromatin and compressing the cell, which leads to cell shrinkage and death (37). Several studies indicate that the toxic effect on cancer cells results from the active Physico-chemical interaction of silver atoms with cellular proteins for functional groups in cells, as well as with nitrogenous bases and phosphate groups in DNA, which causes cell death induced by DNA damage (38).

It has been observed that the toxicity of cancer cells is greater than that of normal uninfected cells. Therefore, using silver nanoparticles is a typical therapeutic strategy (39).

Decreased MI and BI indices occur as a result of cell death or cessation at an interphase stage and the effect of silver nanoparticles on its division, which may be due to the effect of the toxin that binds to cell receptors located in the plasma membrane and leads to the sensitization process and responds to these substances and stimulates the systems responsible for the detoxification process (40). The effect of any cleaved material is occurred by activating the genetic material of the cell, as growth genes are activated to command the entry into the mitotic cycle and within a specific period that depends on the cell type and the efficiency of the stimulating material in the presence of silver nanoparticles, the cell will not be able to go through its four phases during the normal division period and may have exceeded the first mitotic cycle (M1), however, will not be able to pass in the second and third ones (41).

5. Conclusions

The present study demonstrated the synthesis of silver nanoparticles using locally *L. gasseri*. The synthesized silver nanoparticles were characterized by using UV-visible spectrophotometer, FE-SEM, and Fourier

Transform Infrared Measurement (FTIR). The antioxidant activity of AgNPs was observed. Silver nanoparticles exhibited cytotoxic activity against SK-GT-4 cancer cells and do not affect normal cells.

Authors' Contribution

Study concept and design: R. A. J.
Acquisition of data: N. N. H.
Analysis and interpretation of data: R. A. J.
Drafting of the manuscript: R. A. J.
Critical revision of the manuscript for important intellectual content: R. A. J. and N. N. H.
Statistical analysis: N. N. H.
Administrative, technical, and material support: N. N. H.

Conflict of Interest

The authors declare that they have no conflict of interest.

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References

1. Williams NT. Probiotics. Am J Health Syst Pharm. 2010;67(6):449-58.
2. Saleh G. Isolation and Characterization of Unique Fructophilic Lactic Acid Bacteria from Different Flower Sources. Iraqi J Agric Sci. 2020;51:508-18.
3. Mohammed MJ, Mahdi MS, Jameel AH, Thalj KM, editors. The Role of Lactobacillus Casei and Lactobacillus Acidophillus to Decrease the Biological Effects of Potassium Bromate in Rats. 2021.
4. Alexander JW. History of the medical use of silver. Surg Infect (Larchmt). 2009;10(3):289-92.
5. Rajeshkumar S, Bharath LV, Geetha R. Chapter 17 - Broad spectrum antibacterial silver nanoparticle green synthesis: Characterization, and mechanism of action. In: Shukla AK, Iravani S, editors. Green Synthesis, Characterization and Applications of Nanoparticles: Elsevier; 2019. p. 429-44.
6. Tyagi P. Production of Metal Nanoparticles from Biological Resources. Int J Curr Microbiol Appl Sci. 2016;5:548-58.
7. Maeh RK, Jaaffar AI, Al-Azawi KF. Preparation of Juniperus extract and detection of its antimicrobial and antioxidant activity. Iraqi J Agric Sci. 2019;50(3):1153-61.
8. Jubeir S, Ahmed W, Mohammed S. Effect of nanofertilizers and application methods on vegetative growth and yield of date palm. Iraqi J Agric Sci. 2019;50:267-74.
9. Anandalakshmi K, Venugobal J, Ramasamy V. Characterization of silver nanoparticles by green synthesis method using Pedalium murex leaf extract and their antibacterial activity. Appl Nanosci. 2016;6(3):399-408.
10. Pugazhendhi A, Prabakar D, Jacob JM, Karuppusamy I, Saratale RG. Synthesis and characterization of silver nanoparticles using Gelidium amansii and its antimicrobial property against various pathogenic bacteria. Microb Pathog. 2018;114:41-5.
11. Atwan QS, Hayder NH. Eco-friendly synthesis of Silver nanoparticles by using green method: Improved interaction and application in vitro and in vivo. Iraqi J Agric Sci. 2020;51:201-16.
12. Al-Ziaydi AG, Al-Shammari AM, Hamzah MI, Kadhim HS, Jabir MS. Hexokinase inhibition using D-Mannoheptulose enhances oncolytic newcastle disease virus-mediated killing of breast cancer cells. Cancer Cell Int. 2020;20:420.
13. Dakhil AS. Biosynthesis of silver nanoparticle (AgNPs) using Lactobacillus and their effects on oxidative stress biomarkers in rats. J King Saud Univ Sci. 2017;29(4):462-7.
14. Tolaymat TM, El Badawy AM, Genaidy A, Scheckel KG, Luxton TP, Suidan M. An evidence-based environmental perspective of manufactured silver nanoparticle in syntheses and applications: a systematic review and critical appraisal of peer-reviewed scientific papers. Sci Total Environ. 2010;408(5):999-1006.
15. Riaz Rajoka MS, Mehwish HM, Zhang H, Ashraf M, Fang H, Zeng X, et al. Antibacterial and antioxidant activity of exopolysaccharide mediated silver nanoparticle

synthesized by Lactobacillus brevis isolated from Chinese koumiss. Colloids Surf B Biointerfaces. 2020;186:110734.
16. Jabir MS, Saleh YM, Sulaiman GM, Yaseen NY, Sahib UI, Dewir YH, et al. Green Synthesis of Silver Nanoparticles Using Annona muricata Extract as an Inducer of Apoptosis in Cancer Cells and Inhibitor for NLRP3 Inflammasome via Enhanced Autophagy. Nanomaterials (Basel). 2021;11(2).
17. Khashan KS, Abdulameer FA, Jabir MS, Hadi AA, Sulaiman GM. Anticancer activity and toxicity of carbon nanoparticles produced by pulsed laser ablation of graphite in water. Adv Nat Sci: Nanosci Nanotechnol. 2020;11(3):035010.
18. Al-Ziaydi AG, Hamzah MI, Al-Shammari AM, Kadhim HS, Jabir MS. The anti-proliferative activity of D-mannoheptulose against breast cancer cell line through glycolysis inhibition. 2020;2307(1):020023.
19. Al-Ziaydi AG, Al-Shammari AM, Hamzah MI, Kadhim HS, Jabir MS. Newcastle disease virus suppress glycolysis pathway and induce breast cancer cells death. Virusdisease. 2020;31(3):341-8.
20. Al-Salman HNK, Ali ET, Jabir M, Sulaiman GM, Al-Jadaan SAS. 2-Benzhydrylsulfinyl-N-hydroxyacetamide-Na extracted from fig as a novel cytotoxic and apoptosis inducer in SKOV-3 and AMJ-13 cell lines via P53 and caspase-8 pathway. Eur Food Res Technol. 2020;246(8):1591-608.
21. Khashan KS, Sulaiman GM, Hussain SA, Marzoog TR, Jabir MS. Synthesis, Characterization and Evaluation of Anti-bacterial, Anti-parasitic and Anti-cancer Activities of Aluminum-Doped Zinc Oxide Nanoparticles. J Inorg Organomet Polym Mater. 2020;30(9):3677-93.
22. Kareem SH, Naji AM, Taqi ZJ, Jabir MS. Polyvinylpyrrolidone Loaded-MnZnFe 24. Magnetic Nanocomposites Induce Apoptosis in Cancer Cells Through Mitochondrial Damage and P 53 Pathway. J Inorg Organomet Polym Mater. 2020:1-15.
23. Al-Shammari AM, Al-Saadi H, Al-Shammari SM, Jabir MS. Galangin enhances gold nanoparticles as anti-tumor agents against ovarian cancer cells. 2020;2213(1):020206.
24. Verma R, Babu A. Human chromosomes. Manual of basic techniques. Jpn J Hum Genet. 1990;35(1):117-.
25. Sameen AM, Jabir MS, Al-Ani MQ. Therapeutic combination of gold nanoparticles and LPS as cytotoxic and apoptosis inducer in breast cancer cells. 2020;2213(1):020215.
26. Mallikarjuna K, Narasimha G, Dillip GR, Praveen B, Shreedhar B, Lakshmi CS, et al. Green synthesis of silver nanoparticles using Ocimum leaf extract and their characterization. Dig J Nanomater Biostructures. 2011;6(1):181-6.
27. Sikder M, Lead JR, Chandler GT, Baalousha M. A rapid approach for measuring silver nanoparticle concentration and dissolution in seawater by UV-Vis. Sci Total Environ. 2018;618:597-607.
28. Zhao X, Yan L, Xu X, Zhao H, Lu Y, Wang Y, et al. Synthesis of silver nanoparticles and its contribution to the capability of Bacillus subtilis to deal with polluted waters. Appl Microbiol Biotechnol. 2019;103(15):6319-32.
29. Konop M, Damps T, Misicka A, Rudnicka L. Certain Aspects of Silver and Silver Nanoparticles in Wound Care: A Minireview. J Nanomater. 2016;2016:7614753.
30. Sulaiman GM, Al-Amiery AA, Bagnati R. Theoretical, antioxidant and cytotoxic activities of caffeic acid phenethyl ester and chrysin. Int J Food Sci Nutr. 2014;65(1):101-5.
31. Tan LV, Tran T, Thi VD. Biosynthesis of Silver Nanoparticles from Bacillus licheniformis TT01 Isolated from Quail Manure Collected in Vietnam. 2021;9(4):584.
32. Nehia NH, Amen HM. Detection of the antibacterial activity of AGNPS Biosynthesis by Pseudomonas aeruginosa. Iraqi J Agric. 2019;50(2):617-25.
33. Bedlovicova Z, Strapac I, Balaz M, Salayova A. A Brief Overview on Antioxidant Activity Determination of Silver Nanoparticles. Molecules. 2020;25(14).
34. Swamy MK, Akhtar MS, Mohanty SK, Sinniah UR. Synthesis and characterization of silver nanoparticles using fruit extract of Momordica cymbalaria and assessment of their in vitro antimicrobial, antioxidant and cytotoxicity activities. Spectrochim Acta A Mol Biomol Spectrosc. 2015;151:939-44.
35. Sulaiman GM, Hussien HT, Saleem MMNM. Biosynthesis of silver nanoparticles synthesized by Aspergillus flavus and their antioxidant, antimicrobial and cytotoxicity properties. Bull Mater Sci. 2015;38(3):639-44.
36. Buttacavoli M, Albanese NN, Di Cara G, Alduina R, Faleri C, Gallo M, et al. Anticancer activity of biogenerated silver nanoparticles: an integrated proteomic investigation. Oncotarget. 2018;9(11):9685-705.
37. Nuraeni N, Rilisa C, Permataningtyas DE, Armeina D, Yulianti E, Ali MS. Nano-medicine: The Anti-Cancer Effects of Silver Nanoparticles (AgNPs) by Spirulina platensis as a Novel Adjuvant Therapy of Breast Cancer. 2020.

38. Somayyeh M, Hammed A, Motallebi A, Anvar A, Jafar R-N, Shokrgozar M. Toxicity Study of Nanosilver (Nanocid®) on Osteoblast Cancer Cell Line. Int Nano Lett. 2011;1.
39. Abdel-Fattah W, Ghareib W. On the anti-cancer activities of silver nanoparticles. J Appl Biotechnol Bioeng. 2018;5.
40. Carpenter DO, Arcaro K, Spink DC. Understanding the human health effects of chemical mixtures. Environ Health Perspect. 2002;110 Suppl 1:25-42.
41. Varnosfadrani A, Bagheri M. The Issue of Translating Culture: A Literary Case in Focus. Theory Pract Lang Stud. 2012;2.