Cytotoxic Effect of Silver Nanoparticles Prepared by Biosurfactant Produced from Pathogenic Bacteria

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Abstract

This study was aimed to biosynthesis of silver nanoparticles by using biosurfactant (lipopeptides) produced from local isolate *L. plantarum*isolated from clinical samples of Iraqi healthy women which previous identify grown in natural media (BCDFTM), then studied it cytotoxic effect against MCF-7 cell line. The cytotoxic effect of biosurfactant against MCF-7 cell line using 400 µg/ml reached 61.49 % of growth inhibition which is acceptable from cytotoxicity viewpoint. Silver nanoparticles application in vitro as cytotoxic activity against MCF-7 cell line using 400 µg/ml reached 55.67 % in growth inhibition, while when nanoparticles combined with lipopeptides obtained increase the growth inhibition MCF-7 cell and reached 68.44% at the same concentration of AgNPs.

Keywords: *Biosurfactant,lipopeptide, nano particles, XRD, AFM, TEM*

Introduction

Microbes occupy a strong population in the living world. They possess extra and intracellular vital products such as antibiotics, enzymes, toxins, biopolymers and pigments. More than 10,000 active broad-spectrum metabolites with medicinal properties have been isolated from these microbes [(1)]. However, most of the microbial worlds remain unexplored owing to its vastness. Recent studies confirmed that only <0.1% of microbial world has been investigated till date [(2)]. When listing microbial bioactive compounds, biosurfactants (BSs) are such metabolites with many interesting properties due to their multiple diversities in both structures and functions also with their pronounced usage in industries. BSs are basically amphiphilic surface active agents in bacteria, fungi, and classes of actinomycetes. They belong to classes of glycolipid, glycolipoproteins, glycopeptides, or lipoproteins, lipopeptides or derivatives of fatty acids [(3)], and less likely glycoglycerolipids too [(4)].

Biosurfactant are now mediating new developments in the field of Nanotechnology. It observed that biosurfactant produced by microorganisms could play a very important role in aggregation and stabilization process [(5)]. Biosurfactant use has therefore now emerged as a green alternative for enhancing both nanoparticles synthesis (reducing agent) and stabilization (stabilizing agent). One of the modes of action is through adsorbing onto nanoparticles, surface stabilizing the nanoparticles and prevent of formation subsequent aggregation [(6)]. Silver nanoparticles contain a wide spectrum of inhibition of bacteria and other microorganisms. Numerous possible mechanism of action for anticancer activity of silver NPs can be proposed.

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Cytotoxic activity of the silver NPs might be due to its physiochemical interaction with the intracellular DNA and proteins. Reports have shown that the cytotoxicity might also be due to initiation of apoptosis activated by the caspase-3 enzyme [7]. [8]reported that oxidative stress is one of the crucial mechanisms of cytotoxicity induced by silver NPs.The shape and size are important properties that influence the toxicity of silver NPs by elevating reactive oxygen species [9]. Because of the physicochemical differences, some silver NPs are broken-down in the lysosomes and then the release of silver ions result in oxidative stress. In addition, oxidative stress can also lead to genotoxic stress and even up regulation of p53 gene [10], which is an important lead for the application of nanomaterial as anticancer Nano-medicine, as up regulation of p53 gene initiates apoptosis [11].

The aim of the present work is to synthesis nanoparticles prepared bybiosurfactant produced by *L.plantarum* isolated from vagina and testing its cytotoxic effect against cell line.

Methods

Synthesis of silver nanoparticles:

Silver nitrate (AgNO3, 99%) (Aldrich/Germany) was used in the preparation of the silver nanoparticles from biosurfactant produced by selected isolate that grown on natural medium (BCDFTM). Silver nanoparticles were synthesized according to a method described byMartinez-Gutierrez et al.[12].

Characterization of AgNPs

Characterization was performed using a variety of analytical techniques including :The X-ray diffraction (XRD) was used to determine phase identification, crystals structure, composition and physical properties of the synthesized materials silver nanoparticles (AgNPs) [13]. The AFM was also used to determine surface topography of the silver nanoparticles by SPM-AA300 of angstrom advanced Inc. USA, using AFM contact mode [14]. Transmission Electron Microscopy (TEM) analysis was performed to investigate the size, shape, and morphologyof bare and lipopeptidebiosurfactant stabilized Ag NPs [15].

The biological activity of biosurfactant

In *vitro* method was performed to investigate the possiblecytotoxiceffectofextractedbiosurfactantisolated from selected isolateandbiosynthesizednanoparticles on two cell lines on of them is human breast cancer cell line (MCF-7) and another is a normal cell line such as human normal liver cell line (WRL 68).

Maintenance of cell cultures:MCF-7 and WRL 68 cells were maintained in RPMI-1640 supplemented with 10% fetal bovine serum, 100 unit's/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 [16].

Cytotoxicity Assays: To determine the cytotoxic effect of (biosurfactant produced from natural media (BCDFTM), and the effect of the mixture of both biosurfactant and nanoparticles), the MTT cell viability assay was done using 96-well plates. Cell lines (MCF-7 and WRL 68) were seeded at 1×10^4cells / well. After 24 hrs. or a confluent monolayer was achieved, cells were treated with tested compounds at different concentration (12.5, 25, 50, 100, 200, and 400µg/ml). Cell viability was measured after 24 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 the addition of 130 µL of DMSO followed by 37 °C incubation for 15 min with shaking [16-17].

Statistical Analysis: The obtained data were statically analyzed using an unpaired t-test with Graph Pad Prism 6. The values were presented as the mean ± SEM of triplicate measurements [18].

Results

Noble metal nanoparticles (plasmonic) are distinguished from other nanoparticles such as semiconductor quantum dots, polymeric, and magnetic

nanoparticles by their unique surface Plasmon resonance (SPR). The synthesis of AgNPs was monitored by a color change and UV–Vis spectroscopy. The formation of AgNPs was confirmed by changes in the solution color from colorless to yellow brown . Samples are examined by using the UV–V is spectroscopy and the application of the Nano product as an anticancer agent.

The XRD patterns figure (1) show the distinctive diffraction peaks of AgNPs at 2θ = 27.80°, 32.02°, 35.09°, 46.18 ° and 57.38 ° .These peaks were well matched with standard diffraction data of AgNPs (JCPDS file no. 040783) and attributed to the (110), (333), (111), (200) and (220).

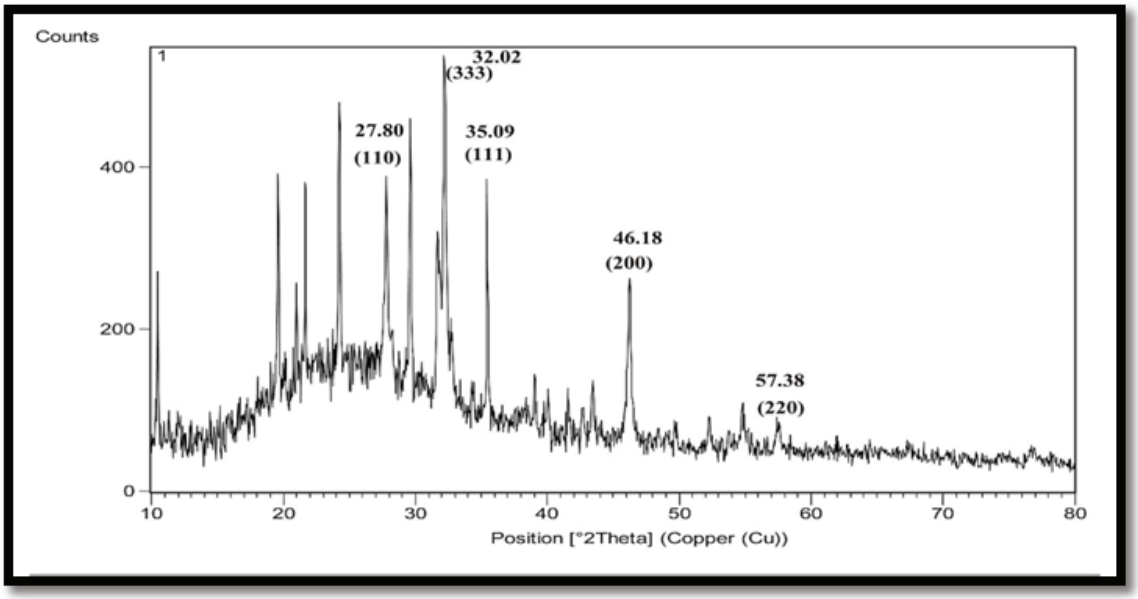

Figure (1) The results of X-ray Diffraction (XRD) for AgNPs.

Surface analysis (AFM) requires good attention because of factors that effect on results such as pollutions. The size of silver nanoparticles was estimated by using AFM-SPM shown in table (1). The result shows that the average size of AgNPs was 52 nm figure (3).

Table (1) Estimation size of AgNPs.

Sample: AA	**Code: Sample Code**
Line No.: lineno	Grain No.:141
Instrument: CSPM	Date:2019-12-08
Avg. Diameter:52.03 nm	<=10% Diameter:0 nm
<=50% Diameter:50.00 nm	<=90% Diameter:60.00 nm

TEM is a valuable tool to analyze the size and morphology of nanoparticles. TEM images of AgNPs as figure (2) showed distributed spherical shaped particles with numerous sizes ranging from 20 to 300 nm.

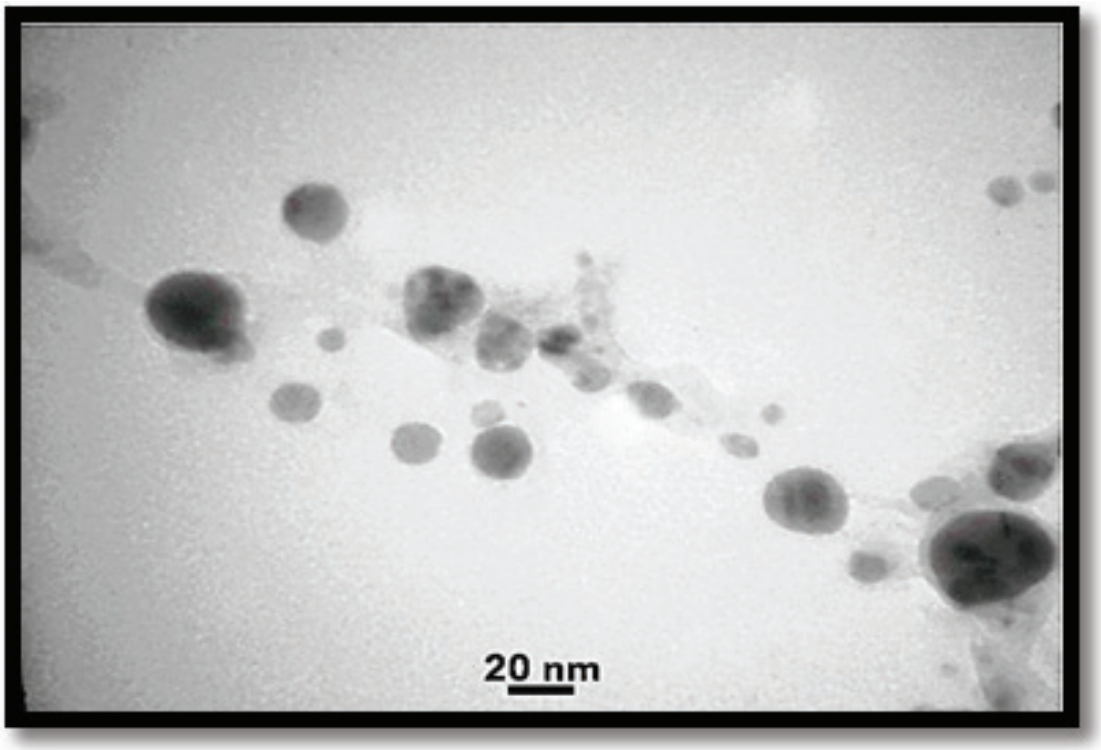

Figure (2) TEM image of silver nanoparticles prepared by biosurfactant.

The biological activity of biosurfactant against cell line.

Results in table (2) show that growth inhibition of REF cell line decreased gradually when biosurfactant concentration increased. biosurfactant has significant differences of cytotoxic effect on MCF-7 cell line ($P \leq 0.05$), 32.67, 54.47 and 61.49 % growth inhibition was showed at concentrations 100, 200, and 400 µg/ml respectively in biosurfactant produced from natural media (BCDFTM).

Table (2) The effect of different concentration of biosurfactant produced from *L. plantarum* grown on BCDFTM on cell inhibition of MCF – 7 cell line.

Concentration of Biosurfactant (BCDFTM) µg/ml	Cell viability (%) by MCF-7	No. of dead cells (%)	Cell viability (%) by WRL68	No. of dead cells (%)
	Mean ± SD		Mean ± SD	
12.5	96.177 ± 1.280	3.82	95.949 ±1.028	4.051
25	95.718 ± 0.810	4.282	95.216 ± 0.821	4.784
50	88.702 ± 3.183	11.298	95.332 ± 1.183	4.668
100	67.329 ± 3.151	32.671	92.438 ± 2.713	7.562
200	45.525 ± 5.157	54.475	82.755 ± 2.842	17.245
400	38.503 ± 4.125	61.497	76.196 ± 2.224	23.804

Significant cytotoxic effect ($P \leq 0.05$) was observed on the growth of MCF-7 cell line at the concentrations of 100, 200 and 400 µg/ml with growth inhibition percentage 25.92, 40.74 and 55.67 % respectively, as shown in the table (3).

Table (3) The effect of different concentration of Nanoparticles produced from biosurfactant grown on BCDFTM on cell inhibition of MCF – 7 cell line.

Concentration of nanoparticles µg/ml	Cell viability (%) by MCF-7	No. of dead cells (%)	Cell viability (%) by WRL68	No. of dead cells (%)
	Mean ± SD		Mean ± SD	
12.5	94.599± 0.837	5.401	94.907 ± 2.199	5.093
25	94.599 ± 2.166	5.401	96.952 ± 1.142	3.048
50	86.921 ± 3.282	13.079	96.180 ± 1.252	3.82
100	74.074 ± 1.819	25.926	92.130 ± 1.557	7.87
200	59.259 ± 5.343	40.741	85.262 ± 0.998	14.738
400	44.329 ± 1.895	55.671	72.068 ± 2.319	27.932

MCF-7 cell line treated with biosurfactant mix with nanoparticles at the same concentrations of 100, 200, and 400 µg/ml and showed growth inhibition percentage of 34.38, 55.75 and 68.44% respectively when used Nano particles with biosurfactant produced from BCDFTMand table (4).Growth inhibition of MCF-7 cell line was increased gradually with the increase of with biosurfactant mix with nanoparticles concentration with significant cytotoxic effect ($P \leq 0.05$) between the concentrations when compared with the control(WRL68).

Table (4) The effect of different concentration of AgNPs mix with same concentration of biosurfactant on cell inhibition of MCF – 7 cell line.

Concentration of AgNPs mix with biosurfactantµg/ml	Cell viability (%) by MCF-7	No. of dead cells (%)	Cell viability (%) by WRL68	No. of dead cells (%)
	Mean ± SD		Mean ± SD	
12.5	95.370 ± 1.863	4.63	94.907± 2.199	5.093
25	94.599 ± 2.166	5.401	96.952 ± 1.142	3.048
50	82.745 ± 1.573	17.255	96.180 ± 1.252	3.82
100	65.617 ± 4.824	34.383	93.463 ± 2.431	6.537
200	44.241 ± 4.492	55.759	84.367± 0.690	15.633
400	31.557 ± 4.634	68.443	77.173 ± 2.278	22.827

Discussion

Biosurfactants usually act as a stabilizing agent of the non-toxic, non-hazardous synthesis of nanoparticles, Biosurfactant use has now emerged as an eco-friendly alternative for enhancing both nanoparticles synthesis and stabilization. Because of the biodegradable nature of biosurfactants, they are more environmentally friendly than chemical surfactants [19].

Biosurfactant produced by microorganisms plays a very important role in aggregation and stabilization process. One of the modes of action is through adsorbing onto metallic nanoparticles, surface stabilizing the nanoparticles and preventing subsequent aggregation. The mechanism of surfactant absorption depends on the type of surfactant and the thickness of the adsorbed layer. So far no comparative study has been published concerning the influence of the biosurfactants nature and composition on the properties and ability to control the production of nanoparticles, Lipopeptidesbiosurfactant has also been reported in nanoparticles synthesis [20].

Clarifiedthat silver with a lattice parameter[21]of a = 4.0862 Å were in good agreement with reference of the face-centered cubic (fcc) crystal lattice of metallic silver, the size of the AgNPs calculated by the Debye-Scherrer equation ($D = 0.94\lambda/d \cos\theta$), and concluded the size of the AgNPs was 38 nm.

Atomic force microscope (AFM) was used to know the surface morphology and to determine topography, the (AFM) gives a two and three-dimensional image of the surface of nanoparticles at an atomic level [21]. The average particle diameter was calculated in nanoscale size. The AgNPs prepared by using biosurfactant were studied using (AFM).

In previous studies, TEM images revealed a size of 30–60 nm spherical-shaped polymeric nanoparticles produced from Cs-Hk fungal cultures, *Streptomyces sp*. MBRC-91and *Bacillus subtilis* MSBN17 [22]. AgNPs with a size around 30–50 nm have been reported for bactericidal activity against various pathogens [23]. Polymeric nanoparticles ranging from 20 to 300 nm have the maximum potential for *in-vivo* applications [24].

Mentionedthat the new therapeutic strategies may be designed by[25], considering that, the use of biosurfactant can alter lipid content to fluidize rigid cancerous tissues and to modulate interfacial properties. While [25] found that the ability of biosurfactant to disrupt cell membranes, leading to a sequence of events that include lysis, increased membrane permeability, and metabolite leakage, have also been suggested as a probable mechanism of antitumor activity. [26] who showed that surfactin induces ROS formation, leading to mitochondrial permeability and membrane potential collapsethat ultimately results in an increase of calcium ion concentration in the cytoplasm afterwards, cytochrome C released from mitochondria to the cytoplasm activates caspase-9 eventually inducing apoptosis. The apoptotic effect induced was associated with a significant decrease in the unsaturated degree of the cellular fatty acids in Bcap-37 cells due to a reduction in the amount of fatty acids, thereby enhancing membrane fluidization [27].

Conclusion

Biosynthesis of AgNPs using produced BS is efficient to convert $AgNO_3$ to spherical shaped particls with numerous size ranging 20 – 100 nm. The result of sytotoxic effect showed that the pofent effect was seen obviously in MCF-7 cell line than WRL68 when used BS and / or AgNPs.

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Conflict of interest: Nil

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