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In Vitro Assessment of the Antioxidant and Antitumor Potentials of Biogenic Silver Nanoparticle

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Abstract

Silver nanoparticles (AgNPs) were biosynthesized using the cell free supernatant of putative probiotic *Lactobacillus paracasei* A26. Several biological activities of biogenic AgNPs were investigated in respect to *in vitro* anti-oxidant and anti-tumor potentials. Anti-oxidant potentials were screened in terms of free radical scavenging activity against two free radicals, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and resazurin dye. AgNPs exhibited a potent scavenging activity against resazurin dye (91±0.046%) with an EC_{50} concentration of 146.823 µg/ml, while scavenging of DPPH was significantly ($P \leq 0.05$) reduced to 72.330±0.114% using a higher EC_{50} concentration of 176.12 µg/ml. The anti-tumor potentials of biogenic AgNPs were studied in relation to the cytotoxicity against two human breast cancer cell lines (CAL-51 and MCF7), using crystal violet dye assay. The viability of AgNPs-treated cancerous cells was significantly decreased in a time- and concentration manner, as compared to insignificant cytotoxic effects against the normal cell line. However, the anti-proliferative activity of AgNPs did not exceed the value of 63.85±0.019% in both cancer cell lines. CAL-51 cells were the most sensitive to the introduced AgNPs, with a maximum decrease in viability of 49.889±0.021% being reached using an IC_{50} value of 98.65µg/ml for 48h exposure time. The inhibition percentage was increased to 60.13±0.005% when the used IC_{50} value was significantly declined to 40.73µg/ml with an exposure time expanded to 72h. MCF7 cells showed lower sensitivity than CAL-51 cells, but with a similar inhibition trend of 59.523±0.01% with an IC_{50} concentration of 66.54 µg/ml for 48h which was increased to 63.857±0.019% when the IC_{50} was reduced to 62.63 µg/ml and the exposure time expanded to 72h. The morphological changes of AgNPs-treated cells were apparent at 72h exposure time, with cells showing apoptotic-like features such as shrinkage and losing of regular fusiform shape. Moreover, cells became detached to surfaces and from each other.

Keywords: Silver nanoparticles, antioxidant agents, DPPH, resazurin dye, cytotoxicity assay, and human breast cancer cell lines

التحري عن الفعالية الضد تأكسدية والضد ورمية لجسيمات الفضة النانوية المحضرة بايولوجيا خارج الجسم الحي

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الخلاصة

انتجت دقائق الفضة النانوية (AgNPs) باستخدام راشح بكتريا *Lactobacillus paracasei* A26 المدعمة للحياة ، وتم التحري عن بعض الأنشطة البيولوجية لدقائق AgNPs المنتجة حيويا في ما يتعلق بالفعاليات المضادة للأكسدة والأورام خارج الجسم الحي. حيث اختبرت الفعالية المضادة للأكسدة بدلالة اختزال الجذور الحرة لكل من:2,2−Diphenyl−1−picrylhydrazyl (DPPH) وصبغة الريزازارين (resazurin dye), أظهرت AgNPs نشاط اختزالي عالي ضد صبغة الريزازارين (91±0.049%) و كانت قيمة EC_{50} 146.823ميكروغرام / مل ، في حين انخفضت النسبة المئوية لاختزال DPPH معنويا ($P\leq0.05$) إلى 72.330±0.114%، وارتفعت قيمة EC_{50} إلى 176.12 ميكروغرام / مل. الفعالية الضد ورمية درست بدلالة السمية الخلوية للدقائق النانوية ضد اثنين من خطوط الخلايا الورمية لاورام الثدي البشرية CAL−51و MCF7 و باستخدام صبغة البلور البنفسجي (Crystal violate) حيث انخفضت حيوية الخلايا الورمية المعاملة بدقائق الفضة النانوية وبشكل ملحوظ واعتمادا على تراكيز الدقائق المستخدمة وفترة التعرض في حين لم تظهر اي آثار سمية تذكر على خط الخلايا الطبيعية ومع ذلك فإن نسب تثبيط حيوية الخلايا الورمية لم يتجاوز 63.85±0.019% و في كلتا خطوط الخلايا الورمية المستخدمة، وكانت خلايا CAL−51هي الأكثر حساسية للدقائق النانوية، حيث كانت النسبة المئوية لاختزال حيوية الخلايا هي 49.889 ± 0.021% وبقيمة IC_{50} 98.65 ميكروغرام / مل عند المعاملة لفترة 48 ساعة، وارتفعت النسبة المئوية لاختزال حيوية الخلايا إلى 60.13 ± 0.005 ٪ ، وفي الوقت نفسه انخفضت قيمة IC_{50} إلى 40.73 ميكروغرام / مل عند ازدياد فترة تعريض الخلايا إلى 72 ساعة، وكانت خلايا MCF7 اقل حساسية للمعاملة بالدقائق النانوية ولكن على نحو مماثل اختزلت حيوية الخلايا و بنسبة 59.523±0.01% و بقيمة IC_{50} 66.54 ميكروغرام / مل عند المعاملة لفترة 48 ساعة ، وارتفعت نسبة تثبيط حيوية الخلايا الى 63.857±0.019% مع زيادة فترة التعريض الى 72 ساعة وانخفضت بالمقابل قيمة IC_{50} إلى 62.63 ميكروغرام / مل . كانت التغيرات المظهرية للخلايا المعالجة بدقائق الفضة النانوية واضحة عند فترة التعريض لمدة 72 ساعة حيث ابدت الخلايا مظاهر شبيهة بمظاهر الموت المبرمج و تقلصت الخلايا وفقدت شكلها المغزلي المميز علاوة على ذلك أصبحت الخلايا غير مترابطة مع بعضها و انفصلت عن اسطح التصاقها.

Introduction

Nanotechnology is a continuously developing area of research covering a number of interdisciplinary fields, such as electronics, medicine and biomaterials. Nanoparticles (NPs) constitute a class of materials with sizes in the range of 1-100 nm, with production stages that involve the design, synthesis and manipulation of structures in particles [1]. Among the commonly used nanoparticles, silver nanoparticles (AgNPs) have an important position because of their distinctive physical-chemical and biological attractiveness [2]. AgNPs have stronger effects than those of silver ions (Ag^{+}) as disclosed in some research. Silver is a noble metal that has been used widely over 200 years to make coins and jewelry. Silver is also resistant to bacteria which renders it a potential low toxic anti-bacterial [3] and anti-fungal agent [4]. The expanding interest on the application of AgNPs in biology and healthcare has raised the need for the development of cheap, feasible and eco-friendly methods for their preparation, such as the green chemistry approaches [5]. Research on AgNPs green biosynthesis using microorganisms have gained significant interest due to its simplicity, low cost of production, and eco-friendliness. The biosynthesis of AgNPs using bacteria is globally well investigated [6]. Probiotics are one of the new and prospect areas of applications in nano biotechnology fields. The probiotic *Lactobacillus* spp. is a promising candidate for AgNPs eco-friendly biosynthesis [7]. Cancer is a disease characterized by uncontrolled cellular growth and spread, during which cells become unresponsive to the usual check-points, leading to tumor growth and metastasis [8]. Nowadays, biogenic AgNPs are gaining much more interest in the field of nano medicine due to their unique properties and obvious therapeutic potential in treating a variety of diseases, including cancer [9].

Cancer is a disease characterized by uncontrolled cellular growth and spread, during which cells become unresponsive to the usual check-points, leading to tumor growth and metastasis [10]. However, chemotherapy does not confer drugs that specifically target cancerous sites and, therefore, exposing healthy cells to undesirable effects. Moreover, a large dose is required owing to its rapid

elimination and nonspecific distribution [11]. For these reasons, the goal of nano medicine is to identify cost-effective molecules that have high specificity and sensitivity in cells. In this approach, AgNPs is a promising tool as anti-tumor agents. Many studies demonstrated results that support the anti-neoplastic properties of AgNPs that can be used as an alternative tool for cancer therapy [12]. Cellular toxicity studies based on the size of AgNPs (about 5-50nm) showed effects in terms of cell morphology, cell survival, the integrity of the cell membrane, oxidative stress (OS) and cell cycle progression in human cell lines [12]. It was suggested that AgNPs can enter cells by endocytosis and that their cytotoxicity occurs in sequential steps. When Ag NPs are subjected to endocytosis, they undergo a degradation process that induces a release of Ag^{+}, causing reactive oxygen species (ROS) generation and glutathione (SGH) level reduction [13]. The augmentation of cellular superoxide radicals triggers alterations in the transmembrane potential of mitochondria and influences signal transduction pathways, which play an important role in apoptosis program activation and cell death [13].

There are only a few locally published documents that study the role of biogenic AgNPs as an antioxidant and antitumor properties. Therefore, the present study aims at screening antioxidant and antitumor potentials of eco-friendly biogenerated AgNPs using the probiotic *Lactobacillus paracasei* A26.

Materials and method

Synthesis of silver nanoparticles (AgNPs)

Silver nanoparticles were biosynthesized using the cell free supernatant (CFS) of putative probiotic *Lactobacillus paracasei* A26 which was supplied by the laboratories of Biology Dep. College of Science / Baghdad University. The biogenic AgNPs were detected and characterized by different analysis techniques such as Ultraviolet-visible spectroscopy (UV-vis spectroscopy); atomic force microscopy (AFM), transmission electron microscope (TEM), X-ray Diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR). The analysis confirmed that the biogenic AgNPs were with an average particle size of 5-10nm (Nano scaled). The generated particles were adapted for the detection of their *in vitro* anti-oxidant and anti-tumor potentials.

Anti-oxidant properties

The antioxidant potential was investigated in terms of free radical scavenging activity by using two methods:

- **2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay**

The efficiency of AgNPs in scavenging DPPH was evaluated quantitatively by evaluating their ability to reduce the purple – colored DPPH solution to yellow [14]. 100µL of various serial concentrations (50 up to 300µg/ml) of AgNPs in deionized water were mixed with an equal volume of freshly prepared DPPH (60µM, in absolute methanol) solution. Then, the mixtures were vortexed and allowed to stand for 30 min in dark at 25°C. The absorbance of the resulting solution was measured at 517nm against blank (methanol), L-ascorbic acid (0.1%) as antioxidant reference, and negative control prepared by mixing 100µL of deionized water with 100µl of DPPH. Assays were performed in triplicate. The percentage of scavenging activity against DPPH was calculated based on control reading using the following formula:

Radical scavenging (%) = [A0 –A1/A0] × 100

where A0 is the absorbance of control, A1 is the absorbance of sample.

The EC_{50} values (the AgNPs concentration that led to 50% reduction of the used reagent) were determined from plot.

- **Resazurin dye scavenging assay**

The ability of AgNPs to scavenge free radicals was also measured depending on the reduction of resazurin dye. AgNPs at different concentrations (50 up to 300µg/ml) were mixed with an equal volume of resazurin dye (1% w/v). Tubes were incubated at 25°C for 15 mins. The absorbance of the mixtures was taken at 600 nm, with the control being prepared by mixing all of the used reagents, except for AgNPs, with distilled water, while L-ascorbic acid (0.1%) was used an antioxidant reference [15]. The percentage of resazurin scavenging was calculated using the same formula as that followed for the scavenging of DPPH.

In vitro cytotoxicity assay of AgNPs

Two human breast cancer cell lines, CAL-51 and MCF-7, were used in the assessment of the potential antitumor activity of biogenic AgNPs. Mouse embryonic fibroblasts (MEF) were used as a

predictive model of normal cells. All cell lines were obtained from the cell culture research laboratory, Iraqi Biotechnology Company, iRAQBiotech (Baghdad, Iraq). The tumor cell lines were maintained in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 1% penicillin /streptomycin and 10% fetal calf serum (FCS), while MEF cells were maintained in Roswell Park Memorial Institute-1640 medium (RPMI-1640) supplemented by penicillin/streptomycin (1%) and 10% FCS. All used media were purchased from Sigma-Aldrich, Germany. The assay was conducted on 96-well microtiter plates [16]. Cell lines were prepared at a concentration of 1×10^5 cells/ml, where 200µl of cell suspension in growth medium were seeded per well. The plates were sealed with a self-adhesive film, lid placed on, and preincubated in 95% humidified atmosphere with 5% CO_2 at 37°C. After 24h or when the confluent monolayer was achieved, i.e. cell exponential growth, the medium was removed and 200µl of various 2-fold concentrations (100, 50, 25, 12.5 and 6.25 µl/ml) of AgNPs in serum-free DMEM were individually seeded to wells. The control was set up as cells treated with 200µl serum-free DMEM and the blank wells contained cell-free medium. The plates were independently incubated for 48h and 72h. Cell viability was measured by replacing AgNPs solution with 200 µl of crystal violet (CV) solution to each well. The plates were reincubated for 20 min under same conditions. Thereafter, CV stain was aspirated, wells were gently rinsed with tap water for several times, and the plates were left at room temperature to dry. Absorbance of each plate was read using ELISA microplate spectrophotometer (Asays, Belgium) at 492 nm. The experiment was carried out in triplicates and the inhibitory rate (IR) was calculated using the following formula [17]:

$$IR\% = [(AT-AB) / (AC-AB)] \times 100$$

where AT represents the absorbance of treated wells, AB represents the absorbance of blank, and AC represents the absorbance of control.

Dose-response curves of % IR versus $\log_{10}$ concentration were constructed and IC_{50} values were determined from plots by interpolation [18]. Inverted phase contrast microscope (Olympus, Japan) was used for the visualization of the morphological alterations induced by AgNPs in MCF-7 and CAL-51 cells treated with the IC_{50} concentration, and the images were captured at 400 X.

Statistical analysis

One-way analysis variance (ANOVA) and t-teat were used for data analysis. All results were expressed as mean ±SD. Differences at $P\leq0.05$ were considered as statistically significant.

Results and discussion

Antioxidant properties

The antioxidant potential of biogenic AgNPs was analyzed by free radical scavenging capacity using two different free radicals, DPPH and resazurin dye, due to the fact there is no universal method to evaluate antioxidant activity and, thus, it is necessary to use different methods to properly evaluate the antioxidant capacity [19]. The efficiency of scavenging DPPH was evaluated quantitatively for AgNPs by their ability to reduce the purple- colored of DPPH solution to yellow [20]. The color changes confirmed that the biogenic AgNPs has antioxidant properties, in comparison to ascorbic acid. Interestingly, AgNPs showed a significant antioxidant potential in a dose-dependent manner against DPPH Figure-1(A). The maximum antioxidant activity (72.330±0.114) was achieved using 300µg/ml, whereas the minimum activity (34.95±0.268%) was related to 50 µg/ml concentration. The antioxidant activity expressed at EC_{50} was 176.12µg/ ml (Figuer-1 C). A strong antioxidant potential of biogenic AgNPs was also observed in the scavenging of resazurin dye in comparison with the reference ascorbic acid. Color change from blue to pink and then colorless indicated the antioxidant activity. The principle of the resazurin assay depends on the change in color of the dye (blue and non-fluorescent) to pink and highly fluorescent when reduced to resorufin. The antioxidant compounds are able to transfer the hydrogen atom to resazurin, leading to the quenching of the color and discoloration of the solution, which is proportional to their amount [21]. Similarly, in a dose-dependent manner, biogenic AgNPs caused the reduction of resazurin to resorufin (Fig.1 B). The highest antioxidant activity (91±0.046%) was achieved at a concentration of 300µg/ml, while the lowest was recorded at 50µg/ml (13.33±0.448%). The EC_{50} value required for the reduction of resazurin (146.823µg/ml) was lower than that for the reduction of DPPH Figure-(1 D).

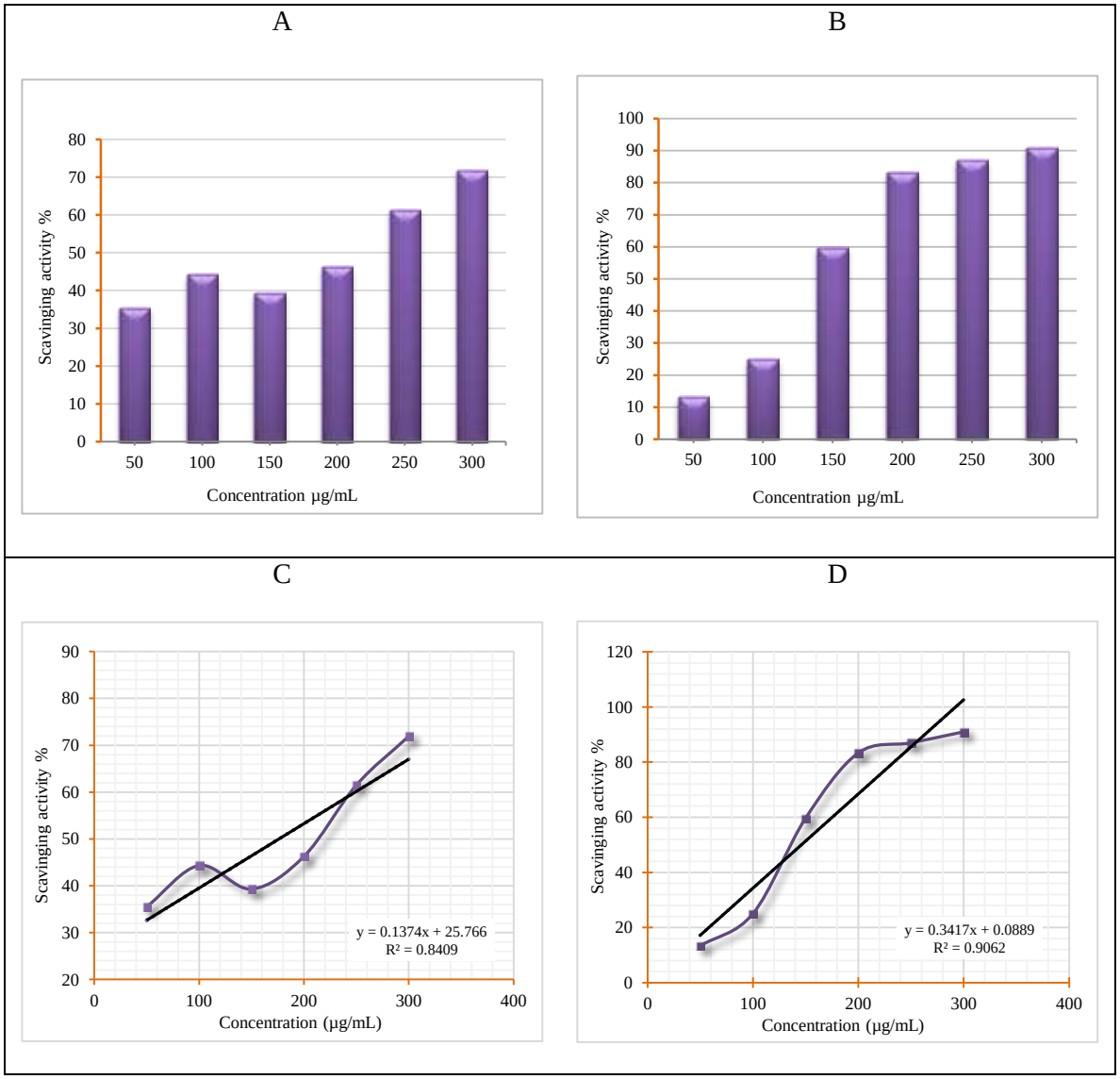


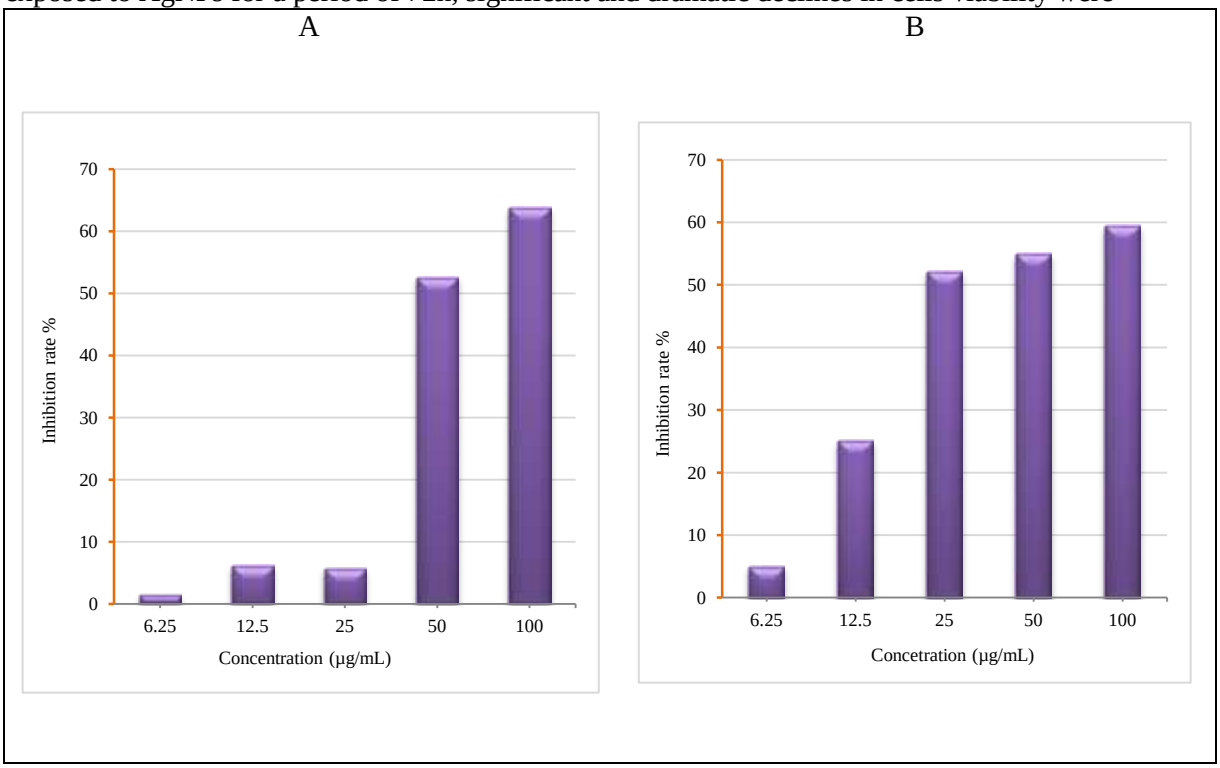
Figure 1-A. DPPH radical scavenging activity of biogenic AgNPs. B. Resazurin dye scavenging activity of biogenic AgNPs. C. Estimated activity of the EC_{50} of biogenic AgNPs for the scavenging of DPPH. D. Estimated activity of the EC50 of biogenic AgNPs for the scavenging of resazurin dye. Each value represents mean ± SD, (n=3).

The free radical scavenging activity of AgNPs was documented, and it was found to be high, due to, high oxidation capabilities, electron loosing and capping agents present on AgNPs surface [22]. The mechanisms of antioxidant activities do not only involve the scavenging of free radicals, but also by inhibiting their production [23]. ROS are formed in the body due to exogenous and endogenous factors and are found to be responsible for many diseases when they are produced at excess levels [24, 25]. Antioxidant compounds have an important role in sequestering ROS from the body, which can decrease the risk of multiple chronic degenerative diseases, including cancer, Alzheimer's, cataract, and coronary heart disease [24, 25]. All biological systems have antioxidant defense mechanisms that protect them against oxidative stress (OS), and repairing enzymes systems fixes the damage caused by free radical attacking. However, this natural antioxidant mechanism can be inefficient due to recent lifestyle, anxious, stress, and environmental contamination that elevates the risk of several diseases [26]. Under constant attack from OS, O_2 in the body splits into singlet or triplet's atoms, which cause damages and mutated cells that are harmful to human health. Hence, external antioxidant compounds from bio-resources were developed to achieve higher specificity, activity, and safety [27]. The high antioxidant activity of biogenic AgNPs recorded in the current study could be due to removing singlet and triplet oxygen, destroying the peroxides, or quenching the free radicals. Moreover, the high

antioxidant activity of AgNPs might be due to the preferential adsorption of biological active compounds from the *L. paracasei* A26 CFS onto the surface of the AgNPs [28].

Cytotoxicity of biogenic AgNPs

CV assay is a method that is useful for screening cell viability and can be used to quantify cell number in culture as a function of absorbance of the dye taken up by the cell. This dye binds to the protein and DNA of the cells. Cells that undergo cell death lose their adherence and are subsequently lost from the population of cells, reducing the amount of dye staining in culture [29]. Two human breast cancer cell lines (CAL-51 and MCF-7), as an *in vitro* model, along with MEF as a normal cell line were used for the evaluation of the antitumor potential (cytotoxicity) of biogenic AgNPs. 2-fold serial concentrations (100, 50, 25, 12.5 and 6.25 µg/ml) of AgNPs were applied with two independent treatment times (48h and 72h). The biogenic AgNPs exhibited significant ($P \leq 0.05$) cytotoxic effects against both of CAL-51 and MCF7 cell lines as compared to the normal cells. The results demonstrated that the viability of AgNPs-treated tumor cells was significantly decreased in time-concentration-response manner, with substantial cytotoxic effects on MEF cells (22.13% maximum viability inhibition at 100 µg/ml conc.). However, cell viability inhibition did not exceed 63.85±0.019% in both tumor cell lines. CAL-51 was the most sensitive cell line to the introduced AgNPs. When MCF7 cells were treated for 48h with various concentrations of AgNPs, cell viability was decreased in a concentration-dependent manner. The incidence of cell death after exposure to 100, 50, 25, 12.5 and 6.25 mg/ml had the values of 1.50±0.07%, 6.17±0.06%, 8.5±0.02%, 52.62±0.01% and 63.85±0.03%, respectively (Figure-2 A), while the value using the IC_{50} was 66.54 g/ml (Fig.3 A). When the exposure time of MCF7 cells to AgNPs was increased to 72h, the AgNPs exerted significant antiproliferative effects as the decrease in cell viability was higher in comparison to that achieved at 48h exposure, except at the highest used concentration (100µg/ml). Whereas, the cell viability decrees were not compatible with previous treatment (Figure-2 B), with no significant ($P \geq 0.05$) difference, as the inhibition percentage was slight less (59.523±0.01%). However, the value of IC_{50} decline to 62.63µg/ml the cytotoxicity value was declined to 62.63µg/ml when the IC_{50} was applied (Fig.3 B). Similarly, AgNPs demonstrated a dose-dependent effect on CAL-51cells. When CAL-51 cells were treated for 48h, cell viability decreased consistently with the decrease in the used concentration; concentrations of 100, 50, 25, 12.5 and 6.25 µg/ml led to cells inhibition values of 3.752±0.096%, 3.973±0.043%, 22.737±0.02 %, 26.931±0.014 %, and 49.9±0.021 % respectively (Figure-2 C), whereas a value of 98.65µg/ml was achieved using the IC_{50} (Figure-3 C). When CAL-51 cells were exposed to AgNPs for a period of 72h, significant and dramatic declines in cells viability were



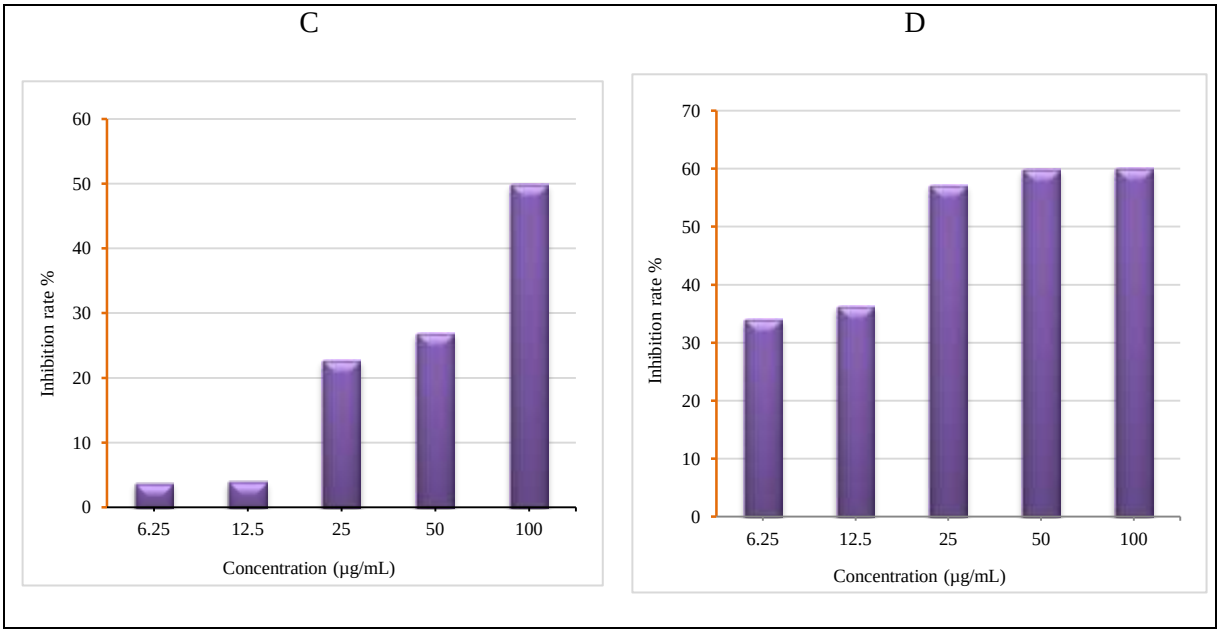


Figure 2-Cytotoxicity of biogenic AgNPs; A. against MCF-7 cell line treated for 48h; B. against MCF-7 cell line treated for 72h; C. against CAL-51 cell line treated for 48h; D. against CAL-51 cell line treated for 72h. Different concentrations (6.25 up to 100 µg/ml) of AgNPs were used. Data are expressed as mean viability ±SD, (n=3).

recognized in most of the used concentrations; loss of cell viability was almost doubled, except when the highest used concentration (100µg/ml) was used which did not lead to a doubled cell inhibition value. Nevertheless, the percentage of inhibition was increased to 60.13±0.005% (Figure-2 D). In addition, the inhibition value using the IC_{50} was significantly declined to 40.73µg/ml (Figure-3 D). These findings revealed the high sensitivity of CAL-51 cells toward biogenic AgNPs, which was higher than that shown by MCF7 cells. The apoptogenic property of AgNPs was additionally investigated through studying the morphological changes in the treated cells under the phase contrast inverted microscope. Images of cells were visualized at 400 X. Such alterations in the cancerous cells were tracked by their treatment with the IC_{50} concentration for two independent time periods (24h and 72h). The resulted morphological alterations are presented in Figure-4

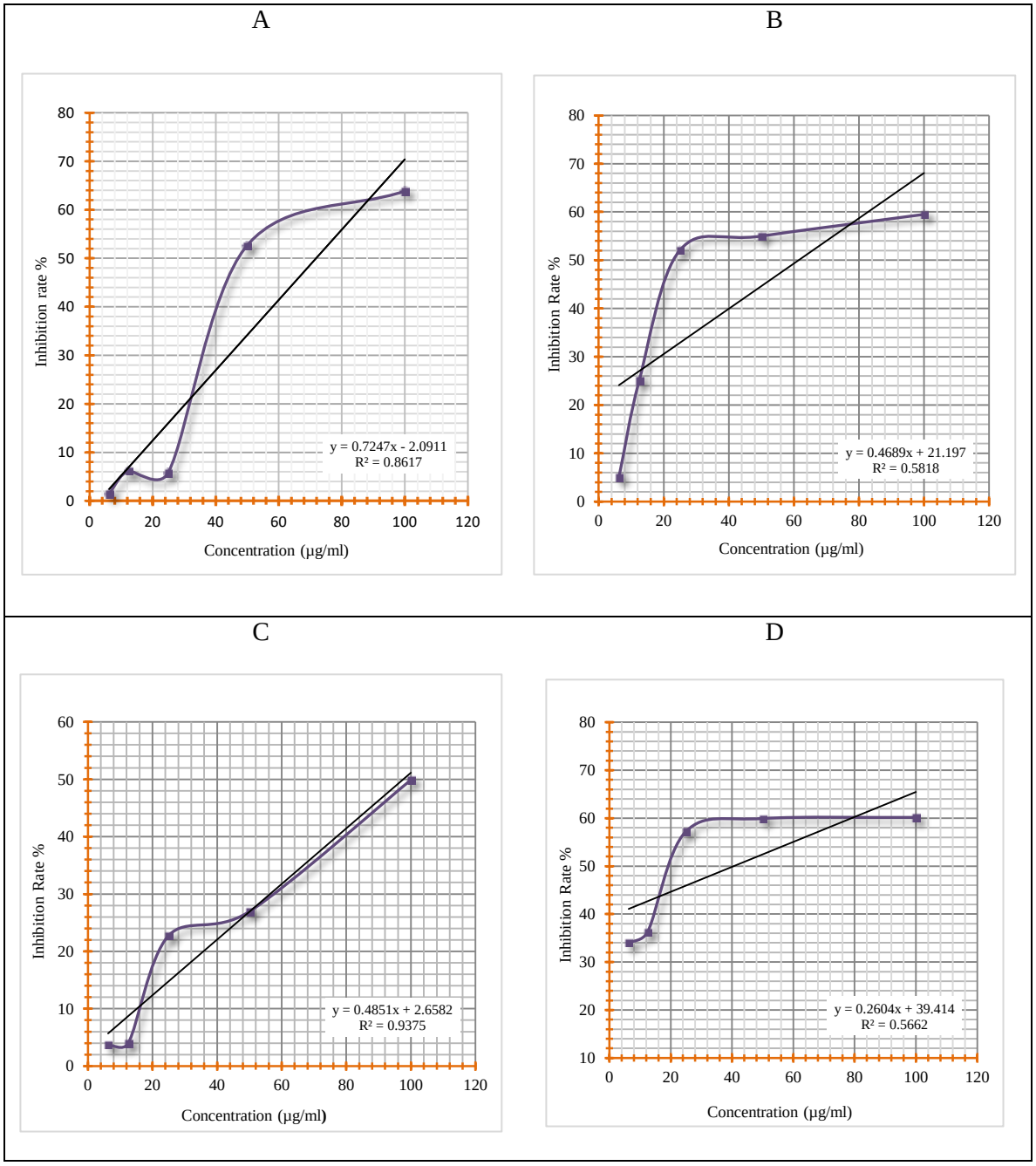


Figure 3-Estimation of the inhibition values of the IC_{50} of biogenic AgNPs; A. against MCF-7 cell line treated for 48h; B. against MCF-7 cell line treated for 72h; C. against CAL-51 cell line treated for 48h; D. against CAL-51 cell line treated for 72h. Different concentrations (6.25-100µg/ml) of AgNPs were applied. Data are presented as mean ± SD, (n=3).

The untreated cells (control) maintained their original morphological form (Figure- 4A), while most of them were adherent to the tissue culture plate and occurred in most images as monolayers. In contrast, obvious morphological alterations were recognized in AgNPs-treated cells. The morphological changes were clearly apparent at 72h exposure time. The treated cells showed apoptotic-like features, as they became irregular, shrunk and lost their fusiform regular shape. As related to the adhesion capacity, the cells became detached from the surface and from each other. Cell density was significantly decreased. Moreover, cytoplasmic blabbing was clear in both cell types.

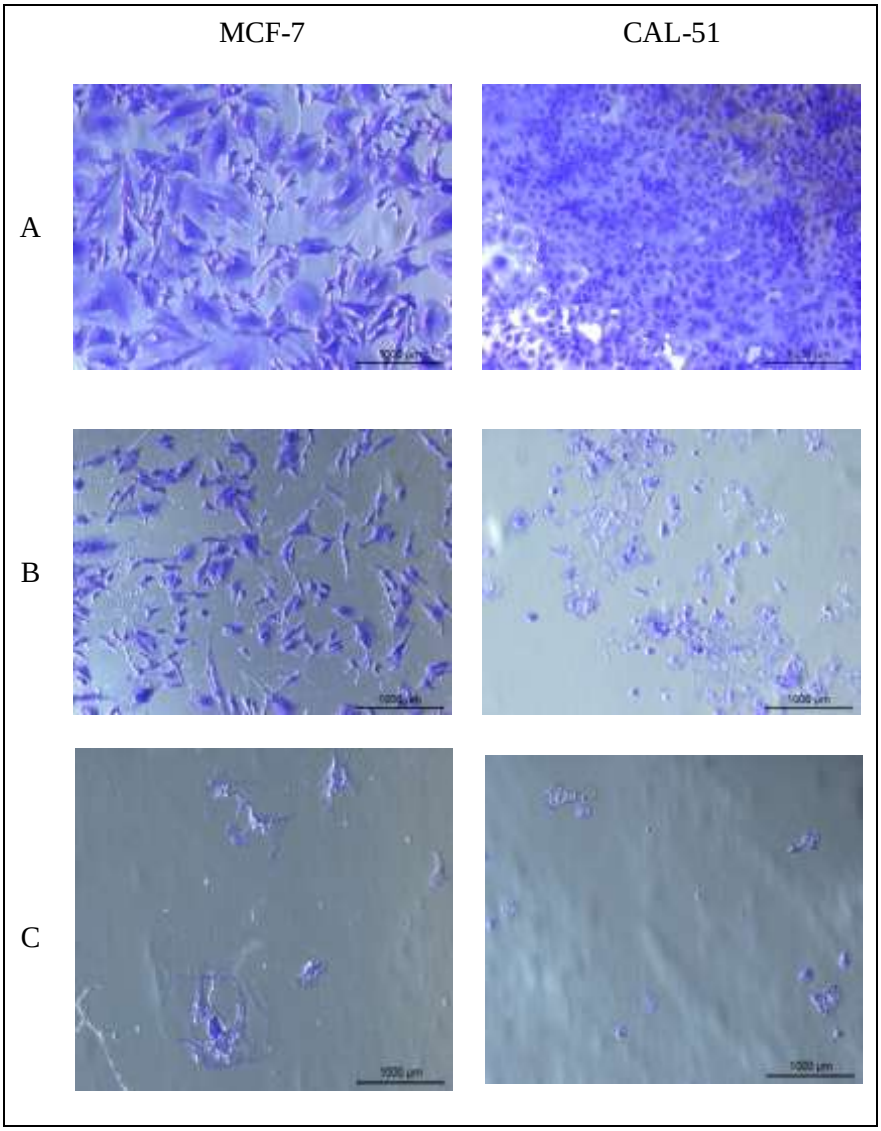


Figure 4-Morphological alterations of MCF-7 and CAL-51 cells. Cells were treated with IC_{50} concentration of biogenic AgNPs for 48 h and 72 h. Images were taken using inverted phase contrast microscope, at 400 X magnification, vs. control. (A) Control cells (B) cells treated for 48 h (C) cells treated for 72 h.

Breast cancer cell lines were used extensively in basic research and provided valuable insights into many aspects of breast cancer biology, including their use as new therapeutic targets for breast carcinoma [30]. The application of AgNPs in cancer research was reported on different cancer cell lines. Cytotoxic potentials of biosynthesized AgNPs on breast cancer cell lines were also investigated. All reported NPs exerted selective cytotoxicity on cancer cells rather than normal cells, in a concentration-dependent manner [31]. In the present study, the cytotoxicity assay showed a significant antiproliferative impact of the adopted AgNPs on both of the targeted tumor cell lines, with minimal toxicity to normal cells (MEF), which agrees with previously published data [32]. It is proposed that the superior cytotoxicity of AgNPs against cancerous cells occurs owing to the highest uptake of NPs by these cells as compared to the healthy cells, given that cancerous cells have an abnormal metabolism and high proliferation rate, which in turn makes them more vulnerable [32]. AgNPs cytotoxicity was reported to be both size and dose-dependent. Particularly, the cellular uptake and toxicity are regulated by size, where smaller particles easily penetrated the cells, while larger particles such as 100nm particles did not [33]. The potent anti-tumor effects of the biogenic AgNPs, which were previously found to be mediated by L. paracasei A26, may be due to the spherical shape and smaller particle size (5-10 nm) of our synthesized particles, as confirmed by different analysis

approaches. On the other hand, the toxicity of AgNPs is not only size-dependent, but it is also related to the type of surface capping groups and particle morphology [34]. Fungal extract-capped AgNPs showed more toxicity than bacterial extract-capped AgNPs in human breast cancer cells [34]. Cytotoxic assays and AgNPs antiproliferative activity are expressed as IC_{50} values. The calculated IC_{50} values showed that the cytotoxicity following 72 hours of exposure was much higher than that of 48h. Also, the estimated values in the present study were much higher than those of the earlier findings. For instance, the IC_{50} value of plant derived AgNPs for MCF-7 cells were reported to be 5 µg/mL, at which a 50% cell death was observed [35]. Nevertheless, the study estimated values of IC_{50} fall within the clinically acceptable concentration [36], particularly for CAL-51 cells, which exhibited less IC_{50} value (40.3 µg/ml), and this is a promising result and within acceptable limits. AgNPs-treated cancerous cells sensitivity depends on cell types [37]. The sensitivity difference between the two cells lines included in the present study may be attributed to the type of these neoplastic cells, each of them has unique recognizable phenotypes and clinical outcomes. Hence, CAL-51 is triple-negative breast cancer (TNBC), estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and Her2-negative, and has higher rates of distant metastasis aggressive cancer [38]. Thus, the effectiveness of the biogenic AgNPs on CAL-51 cells is of high relevance. While, MCF7 cells express both ER and PR, whereas they are Her2-negative [39]. Each therapeutic target for breast carcinoma is largely based on its unique biologic features, availability, and often ER, PR and HER2 status. The cytotoxic behavior of AgNPs is due to their large surface to volume ratio and, thus, they could easily enter the cells, interact with cell constituents, and thereby disturb the cellular signaling pathways [40]. The mechanisms of AgNPs cytotoxicity involve the cellular up taking of NPs via macropinocytosis and clathrin-dependent endocytosis. Various studies revealed that AgNPs act via triggering the intracellular ROS to prevent the antioxidants. The immediately formed ROS then damages DNA and results in cell death [41]. Earlier studies depicted that the cytotoxicity of biogenic AgNPs is related to the involvement of the level of cellular ROS and mitochondrial membrane disruption [42]. Studies of green biosynthesized AgNPs concluded that they induce OS, leading to the apoptosis via caspase-mediated and mitochondria-dependent pathways [43]. The caspase-3-activation cascade plays an important role in several apoptotic mechanisms [44]. The study suggests that the AgNPs induced apoptosis in MCF 7 in a caspase-3-dependent manner, by activating and increasing the levels of this mediator, as previously described [45].

Conclusions

The synthesized AgNPs in the present study revealed antioxidant potential as shown by free radical scavenging behavior. This activity has probably occurred due to the presence of bio-compounds on the surface of AgNPs, which were accepted by the biomolecules of the capping from CFS of *L. paracasei A26*. The findings of the current study suggest an *in vitro* anti-tumor potential of biogenic AgNPs against human breast cancer cells, with high sensitivity of CAL-51 (TNBC) cells to the adopted AgNPs treatments. This finding can be considered as a clinical concern due to aggressiveness and high rate metastatic features of TNBC. By inducing apoptotic-like cells death, these NPs could play a role in the development of new therapeutic agents for the treatment of cancer, or they can act as adjuvants to increase the effectiveness of available therapies.

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