BIOSYNTHESIS OF SILVER NANOPARTICLES USING FUNGI AND BIOLOGICAL EVALUATION OF MYCOSYNTHESIZED SILVER NANOPARTICLES

ABSTRACT:
Eight fungal species were screened for mycosynthesis of silver nanoparticles (AgNPs), by visual observation of fungal filtrate only six fungal species were found to reduce the silver salt into silver nanoparticles. The UV-visible spectra of the biosynthesized nanoparticles (AgNPs) by *Aspergillus ochraceus* (RCMB 036254) cell filtrate showed characteristic surface plasmon absorption at 420 nm. Transmission electron microscopy (TEM) micrograph showed the formation of spherical AgNPs ranging from 5.5 to 24.5 nm diameter. The Qualitative, as well as quantitative status of elemental silver was characterized by Energy Dispersive Analysis of X-ray (EDX). The optimum conditions for maximum production of AgNPs were obtained using 0.75 mM silver nitrate at 40°C and pH 6. The antimicrobial activity of the mycosynthesized nanoparticles under optimum conditions were investigated alone and in combination with commonly used antibiotics against Methicillin-resistant *Staphylococcus aureus* (MRSA). Out of thirteen commonly used antibiotics, the antibacterial efficiency of only five antibiotics has been increased as a result of combination with AgNPs. Biosynthesized silver nanoparticles showed dose-dependent antitumor activity with IC50 at 1.4, 2.1, and 1.2 µg/ml against human colon carcinoma, human breast cancer and human hepatocellular carcinoma cells, respectively, while 39.6 µg/ml was required to induce 50% of normal Vero cell mortality.

KEY WORDS: Biosynthesis, silver nanoparticles, mycosynthesized silver nanoparticles

ARTICLE CODE: 01.02.14

INTRODUCTION:
The prefix nano is derived from the Greek word *Nanos* refers to things of one billionth \((10^{-9} \text{m})\) in size (Narayanan and Sakhthivel, 2010). A nanoparticle is defined as having one dimension 100 nm or less in size. Environmentally toxic or biologically hazardous reducing agents are typically involved in the chemical synthesis of nanoparticles (Ghorbani et al., 2011).

There has been a search for greener production alternatives of metal nanoparticles (Abou El-Nour et al., 2010) many researches have shown that microorganisms, plant extracts, and fungi can produce nanoparticles through biological pathways (Ahmed et al., 2003; Abou El-Nour et al., 2010; Popescu et al., 2010).

Both unicellular and multicellular organisms are known to produce inorganic materials either intra- or extracellularly (Shankar et al., 2004). The ability of microorganisms like bacteria and fungi to control the synthesis of metallic nanoparticles is employed in the search for new materials. Biosynthesis of nanoparticles of different elements is reported from both pathogenic and non-pathogenic fungi (Vigneshwaran et al., 2007).

The fungal systems or myco-nanofactories have been exploited for the synthesis of metal nanoparticles of silver, gold, zirconium, silica, titanium, iron (magnetite) and platinum. A large number of
fungal strains are capable to synthesize silver nanoparticles (AgNPs) extracellularly, among which *Fusarium oxysporum* (Ahmad et al., 2003), *Aspergillus fumigatus* (Bhainsa and D’Souza, 2006), *Aspergillus niger* (Gade et al., 2008), *Fusarium semitectum* (Basavaraja et al., 2008), *Penicillium brevicompactum* (Shaligram et al., 2009), *Cladosporium cladosporioides* (Balaji et al., 2009), and *Aspergillus clavatus* (Verma et al., 2010) have been previously described. Fungi are more advantageous compared to other microorganisms in many ways. Fungal mycelial mesh can withstand flow pressure and agitation and other conditions in bioreactors or other chambers compared to plant materials and bacteria. These are fastidious to grow and easy to handle and easy for fabrication. The extracellular secretions of reductive proteins are more and can be easily handled in downstream processing. And also, since the nanoparticles precipitated outside the cell is devoid of unnecessary cellular components, it can be directly used in various applications (Narayanan and Sakhthivel, 2010).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major nosocomial pathogens responsible for a wide spectrum of infections, including skin and soft tissue infections, pneumonia, bacteremia, surgical site infections (SSI), catheter related infections (de San et al., 2007). Intensive care unit characteristically has higher rates of infections and increased transmission rates, high antibiotic use and large numbers of vulnerable patients. The emergence of bacterial resistance to antibiotics and its dissemination, however, are major health problems, leading to treatment drawbacks for a large number of drugs (Schito, 2008). Consequently, there has been increasing interest in the use of inhibitors of antibiotic resistance for combination therapy (Gibbons, 2008).

It has been demonstrated that AgNPs have effective antimicrobial activity (Aymoner et al., 2002; Sondi and Salopek-Sondi, 2004; Baker et al., 2005; Melaye et al., 2005; Lok et al., 2006). AgNPs have been applied to a wide range of health care products, such as burn dressings, scaffold, water purification systems and medical devices (Thomas et al., 2007).

In vitro cytotoxicity testing procedures reduce the use of laboratory animals and hence use of cultured tissues and cells have increased (Byrd et al., 2000). The discovery and identification of new antitumor drug with low side effects on immune system has become an essential goal in many studies of immuno-pharmacology, it is a challenge to find drugs for the effective treatment of various types of cancers (Xu et al., 2009).

In the present study, we attempted to evaluate antibacterial and antitumor activities of mycosynthesized AgNPs; evaluation of the antibacterial activity singly and in combination with commonly used antibiotics against Methicillin-resistant *Staphylococcus aureus* (MRSA); while antitumor activity was evaluated against different cancer cell lines in vitro.

**MATERIAL AND METHODS:**

**Fungal species:**

Fungal organisms namely, *Aspergillus fumigatus* (RCMB 02568), *Aspergillus flavus* (RCMB 02426) *Candida albicans* (RCMB 05031), *Penicillium italicum* (RCMB 03924), *Syncephalastrum racemosum* (RCMB 05922), *Fusarium oxysporum* (RCMB 08213), *Alternaria solani* (RCMB 07324) and *Aspergillus ochraceus* (RCMB 036254); kindly provided from the Regional Centre for Mycology and Biotechnology, Al-Azhar university, Egypt were screened for production of silver nanoparticles AgNPs.

**Biomass preparation:**

Fungi were grown on malt extract broth at 28°C on a rotary shaker (120 rpm) for 96 h. The biomasses were harvested by filtration using Whatman filter paper No. 1, followed by washing with distilled water to remove any components of the medium. The biomass (25 g) wet weight was placed in individual flasks containing 100 ml water and incubated for 24 h. The biomass was filtered, and the cell filtrate was collected and used for biosynthesis of AgNPs (Devi and Joshi, 2012).

**Biosynthesis of AgNPs:**

For biosynthesis of AgNPs, 50 ml of cell filtrate was mixed with 10 ml AgNO₃ solution (1mM) and reaction mixture without AgNO₃ was used as control. The prepared solutions were incubated at 28°C for 24 h. All solutions were kept in dark to avoid any photochemical reactions during the experiment. The AgNPs were purified by centrifugation at 10,000 rpm for 10 min twice, and collected for further characterization (Devi and Joshi, 2012).

**Characterization of AgNPs:**

After 24 hours of synthesis, the sample of AgNPs was centrifuged at 14,000 rpm for 30 minutes at room temperature. Repeated rinses were performed to remove impurities. The residue of AgNPs was re-suspended in 1 ml sterile water. The production of AgNPs in aqueous solution was monitored at the Regional Centre for Mycology and Biotechnology (RCMB) using:

i. **UV-visible Spectroscopy Analysis:**

Change in colour of the cell free filtrate incubated with silver nitrate solution was visually observed over a period of time. Absorption measurements of the filtrate were...
carried out after 24 h. using UV-visible spectrophotometer (Milton-Roy Spectronic 1201). UV-Visible analysis of several weeks old samples was also carried out to check the stability of synthesized AgNPs (Ingle et al., 2008).

ii. Transmission Electron Microscopy (TEM):

For TEM analysis, a drop of the cell filtrate was placed on the carbon coated copper grids and dried by allowing water to evaporate at room temperature. Electron micrographs were obtained using GEOL GEM-1010 transmission electron microscope at 70 kV (Jain et al., 2011).

iii. Energy Dispersive Analysis of X-ray (EDX):

The presence of elemental silver was confirmed through EDX. The EDX microanalysis was carried out by X-ray micro-analyzer (Oxford 6587 INCA) attached to JEOL JSM-5500 LV scanning electron microscope at 20 kV. The EDX spectrum recorded in the spot profile mode from one of the densely populated silver nanoparticles region on the surface of the film. The nano crystallites were analyzed using Quanta 200 FEG (Devi et al., 2012).

Effect of reaction parameters on the production of AgNPs:

To obtain the optimized reaction parameters giving maximum synthesis of AgNPs, firstly, AgNO\(_3\) ranging from 0.25 to 7 mM (final concentration) was added to the fungal filtrate and incubated for up to 120 hours. After this, AgNO\(_3\) was added to the fungal filtrate and incubated at (5°C–50°C) and at pH (2-10) for temperature and pH optimization, respectively. Then UV-Vis spectra were carried out to study the production with varying reaction parameters.

The antimicrobial activity of AgNPs:

The antibacterial activity of synthesized AgNPs was investigated against Methicillin-resistant *Staphylococcus aureus* (MRSA). The overnight grown MRSA culture was plated on Muller-Hinton agar (MHA). Wells were cut on the plates using cork porer and 50 µl of AgNP solution was dispensed in each well. The mycelia-free water extract alone and AgNO\(_3\) were used as control. The plates were incubated overnight at 37°C for 24 h. and observed for the presence of zones of inhibition. The minimum inhibitory concentration (MIC) of mycelia-free water extract alone, AgNPs and AgNO\(_3\) were determined by broth microdilution method given by the Clinical Laboratory Standards Institute (CLSI, 2004). Two-fold serial dilutions of AgNPs were made using Muller-Hinton (MH) broth (Roy et al. 2010).

Antibiotic susceptibility:

Thirteen antibiotics, namely, Ampicillin (AM10), Penicillin (P10), Oxacillin (OX1), Amoxicillin (AX25), Amoxicillin/clavulanic acid (AMC30), Cephalothin (KF), Cefoxitin (FOX30), Ceftriaxone (CRO30), Vancomycin (VA30), Amikacin (AK30), Tobramycin (TOB10), Erythromycin (E15) and Ciprofloxacin (CIP5) were used to assay the antibacterial efficiency of commonly used antibiotics alone and their combined effect with extra-cellularly mycosynthesized AgNPs against MRSA using disc agar diffusion method (DAD) on Muller-Hinton agar, according to the guidelines recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2002). To determine the combined effects, each standard paper disc was further impregnated with sub-inhibitory concentration of AgNPs. A single colony of MRSA was grown overnight in Muller-Hinton broth medium on a rotary shaker (200 rpm) at 37°C. The inocula were prepared by diluting the overnight cultures with 0.9% NaCl to a 0.5 McFarland standard and were applied to the plates along with the standard antibiotics alone and that combined with sub-inhibitory concentration of AgNPs. After incubation at 37°C for 24 h., the zones of inhibition were measured. The assays were performed in triplicates.

The antitumour activity of AgNPs

The activity of AgNPs was assayed on three tumour cell lines and normal Vero cells.

Cell lines:

Human colon carcinoma (HCT-116) cells, Human breast cancer (MCF-7) cells and Human hepatocellular carcinoma (HepG2) cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium components supplemented with 10% inactivated foetal calf serum and 50 µg/ml gentamycin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO\(_2\) and were sub-cultured two to three times a week (Mosmann, 1983).

Evaluation of the antitumor activity:

The antitumor activity was evaluated on MCF-7, HCT-116 and HepG2 cells. The cells were grown as monolayers in growth RPMI-1640 medium supplemented with 10% inactivated foetal calf serum and 50 µg/ml gentamycin. The monolayers of 10,000 cells adhered at the bottom of the wells in a 96-well microtiter plate incubated for 24 h. at 37°C in a humidified incubator with 5% CO\(_2\). The monolayers were then washed with sterile phosphate buffered saline (0.01 M pH 7.2) and simultaneously the cells were treated with 100 µl from different dilutions of the test sample in fresh maintenance medium and incubated at 37°C. A control of untreated cells was made in the absence of the test sample. Six wells were used for each concentration of the test sample. Every 24 h., the observation under the inverted microscope was made. The number of the surviving cells was determined by staining the cells with crystal violet.
(Muthumary, 2007) followed by cell lysis using 33% glacial acetic acid and read the absorbance at 490 nm using ELISA reader (Sun Rise, TECAN, Inc, USA) after well mixing. The absorbance values from untreated cells were considered as 100% proliferation. The number of viable cells was determined using ELISA reader as previously mentioned and the percentage of viability was calculated as \[1 - (\text{OD}_t / \text{OD}_c) \times 100\%\] where \(\text{OD}_t\) is the mean optical density of wells treated with the test sample and \(\text{OD}_c\) is the mean optical density of untreated cells. The 50% inhibitory concentration (IC\(_{50}\))--the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots.

RESULTS AND DISCUSSION:

Biosynthesis of silver nanoparticles (AgNPs):

Out of the eight fungal species screened, only six fungal species; namely Aspergillus fumigatus (RCMB 02568), Candida albicans (RCMB 05031), Penicillium italicum (RCMB 03924), Syncephalastrum racemosum (RCMB 05922), Fusarium oxysporum (RCMB 08213) and Aspergillus ochraceus (RCMB 036254) were found to reduce silver salt into silver nanoparticles by visual observation of the fungal filtrates. These six fungal filtrates exhibited a gradual change to brown colour, clearly indicating the formation of AgNPs. The colour of the culture filtrate with silver nitrate solution changed to intense brown after 24 h of incubation, whereas, the control (without silver nitrate salt) did not exhibit any colour change. Aspergillus ochraceus (RCMB 036254) exhibited the most intense brown colour compared to the other five fungal species (Fig. 1). The colour changes observed can be attributed to the surface plasmon resonance of deposited AgNPs (Mulvaney, 1996).

Characterization of Ag NPs:

- UV-visible spectroscopy analysis:

The UV-visible spectra of Aspergillus ochraceus (RCMB 036254) filtrate treated with the silver nitrate solutions showed characteristic surface plasmon absorption at 280 and 420 nm (Fig. 2). The absorption at 280 nm indicates the presence of tryptophan and tyrosine residues present in the protein, this observation indicates the release of proteins into filtrate that suggests possible mechanisms for the reduction of silver ions present in the solution as demonstrated by Bhainsa and D’Souza (2006). Fungal cell filtrate treated with silver nitrate solution is known to show peak around 420 nm with high absorbance as demonstrated by Ingle et al. (2008), which supports our finding of the peaks observed for absorbance at 420 nm, indicating the bio-synthesis of nanoparticles by A. ochraceus (RCMB 036254).

- Microscopic characterization by TEM:

The data obtained from transmission electron-micrograph showed distinct shape and size of nanoparticles. The particles were spherical in shape with mean of 13.88 ± 4.11 nm (Fig. 3 & Table 1). AgNPs uniformly distributed with some agglomeration which revealed pattern similar to the biosynthesized AgNPs by Kathiresan et al. (2009) and Jain et al. (2011).
Table 1. Statistical measurements AgNP5 ranging from 5.5 – 24.4 nm

<table>
<thead>
<tr>
<th>Statistical function</th>
<th>Distance (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count</td>
<td>53</td>
</tr>
<tr>
<td>Mean</td>
<td>13.88</td>
</tr>
<tr>
<td>Minimum</td>
<td>5.5</td>
</tr>
<tr>
<td>Maximum</td>
<td>24.43</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>4.11</td>
</tr>
</tbody>
</table>

- Energy Dispersive Analysis of X-ray (EDX):

EDX gives qualitative, as well as quantitative status of elements that may be involved in the formation of AgNPs. Figure 4 shows the EDX spectrum recorded in the spot-profile mode. The optical absorption peak is observed at 3KeV, which is typical for the absorption of metallic AgNPs by Magudapathy et al. (2001). Strong signals from the silver atoms are observed, while weaker signals from Na, Si, S, P, Cl, and Ca atoms are also recorded. From the EDX spectrums, it is clear that AgNPs reduced by Aspergillus ochraceus (RCMB 036254) have the weight percentage of silver as 52.91% (Table 2).

![Fig. 4. EDX spectra of Ag NPs. Silver X-ray emission peaks are labeled. Strong signals from the atoms in the nanoparticles are observed in spectrum and confirms the reduction of silver ions to AgNPs](image)

Table 2. The element composition of the AgNPs of EDX spectra

<table>
<thead>
<tr>
<th>Element</th>
<th>Element%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>14.22</td>
</tr>
<tr>
<td>Si</td>
<td>4.93</td>
</tr>
<tr>
<td>P</td>
<td>2.52</td>
</tr>
<tr>
<td>S</td>
<td>4.08</td>
</tr>
<tr>
<td>Cl</td>
<td>14.89</td>
</tr>
<tr>
<td>Ca</td>
<td>6.44</td>
</tr>
<tr>
<td>Ag</td>
<td>52.91</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

Effect of reaction parameters on the production of AgNPs:

Optimization studies revealed the significant effects of concentration of reaction parameters on the rate of bio reduction of silver ions to AgNPs. At a fixed temperature of 28°C, variation in reaction kinetics was observed for the synthesis of nanoparticles by varying the AgNO₃ concentration and reaction time (Fig. 5). Beyond 72 h. of incubation, no further increase in intensity was recorded indicating complete reduction of silver ions by the fungal cell filtrate. Maximum synthesis of nanoparticles occurred at 0.75 mM AgNO₃ in the reaction mixture, followed by 0.5 mM AgNO₃. Highest concentration (7 mM) AgNO₃ showed the least bio-reduction of silver ions to nanoparticles. This can be explained on the basis of enzyme-substrate kinetics; i.e. the active site in the key biomolecule responsible for reduction is already saturated with the silver ions, and no site is available for excess ions to get reduced, hence there is no further increase in synthesis of AgNPs despite the addition of more salt (Singh et al., 2013). As compared with the UV-Vis spectrum obtained for different concentration of AgNO₃ after 72 h., 420 nm was the optimum wavelength for all concentrations (Fig. 6). At the optimized AgNO₃ concentration of 0.7 mM, rate of synthesis was found to increase with an increase in reaction temperature up to 40°C, which showed maximum synthesis (Fig. 7) after which a decline in the synthesis was observed and that may be due to deviations from the optimized parameters resulted in an increase in size and poly dispersity of AgNPs as demonstrated by Singh et al. (2013). pH 6 was found to provide optimal conditions for the maximal synthesis of nanoparticles (Fig. 8). Despite a large number of reports on the synthesis of Ag NPs, only few reports are available on the optimization. One such study was reported by Gurunathan et al. (2009) for E. coli-mediated Ag NPs synthesis, where 5 mM AgNO₃, 60°C temperature, and pH 10 were reported to provide optimal conditions for the maximal synthesis of small sized nanoparticles. In the present study, the enhanced rate of synthesis of AgNPs at optimized conditions might be the direct result of the effect of substrate (silver ions), pH and temperature on a key biomolecule responsible for the reduction present in the aqueous filtrate of A. ochraceus.
Fig. 5. Optimization of AgNO$_3$ concentration for AgNPs synthesis.

Fig. 6. UV-Vis spectra of AgNPs synthesis obtained with different concentrations of AgNO$_3$.

Fig. 7. Optimization of reaction temperature for AgNPs synthesis.
Antibacterial activity of AgNPs:

Silver and its compounds are known for their antimicrobial properties and for the treatment of burns and chronic wounds (Shakibaie et al., 1998). High surface area to volume ratio cause high bactericidal activity of AgNPs compared with bulk silver metal (Cho et al., 2005; Panyala et al., 2008).

It is well known that Ag ions and Ag-based compounds have biological activities (Furno et al., 2004). In the current study silver nanoparticles exhibited moderate antibacterial activity against MRSA better than that of AgNO$_3$ with zone of inhibition of 16.7 mm and 13.2 for AgNPs and AgNO$_3$, respectively, while the fungal extract did not show any activity against MRSA (Table 3). The reason for this is the structural composition of Gram-positive bacteria (Gram-positive bacteria possess a thick layer of peptidoglycan (20–80 nm), making it difficult for AgNPs to penetrate). Owing to their small size, AgNPs impair the sulphur and phosphorus containing essential macromolecules such as proteins and DNA (Wei et al., 2009). Thus, action of AgNPs appears to be a consequence of adherence to and penetration inside the cell of the target cells.

Table 3. Zone of inhibition (mm) and Minimum inhibitory concentration (MIC) of AgNO$_3$, AgNPs and aqueous extract against MRSA

<table>
<thead>
<tr>
<th>Test organism</th>
<th>AgNO$_3$</th>
<th>AgNPs</th>
<th>Aqueous extract</th>
<th>Reference drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA</td>
<td>13.2</td>
<td>16.7</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>MIC (µg/ml)</td>
<td>31.25</td>
<td>15.63</td>
<td>0</td>
<td>62.5</td>
</tr>
</tbody>
</table>

The effect of AgNPs on the antibacterial activity of thirteen antibiotics was investigated against MRSA using disk diffusion method.

The antibacterial resistance of MRSA against various antibiotics decreases with nanoscaled AgNPs. The diameter of inhibition zones (mm) around the different antibiotic discs with and without AgNPs against MRSA are shown in (Table 4 & Figs 9 and 10). It should be pointed out that the AgNPs content of 7.81 µg/ disc was chosen to guarantee that the effect produced was due to the combination and not to the effect of the AgNPs itself. The antibiotic efficacy of some of tested antibiotics has been significantly improved in the presence of nano size silver against MRSA. The highest antibacterial activities increases in area were observed for Ceftriaxone (13 mm) followed by Amoxicillin/clavulanic acid (10 mm) followed by Amikacin and Ciprofloxacin (6 mm in each). The lowest increase in inhibition zone area (3 mm) was reported for Tobramycin. Conversely, Ampicillin, Penicillin, Oxacillin, Amoxicillin, Cephalothin, Cefoxitin, Vancomycin and Erythromycin AgNPs showed no effect on the antibacterial activity of these antibiotics against MRSA. So the effect observed in this condition is due to the antibiotic-AgNPs combination. It is believed that microorganisms carry a negative charge while metal oxides carry a positive charge. This creates an “electrostatic” attraction between the microbe and treated surface. Once the contact is made, the microbe is oxidized and dead instantly. Generally, it is believed that nanomaterials release ions, which react with the thiol group (−SH) of the proteins present on the bacterial surface. Such proteins protrude through the bacterial cell membrane, allowing the transport of nutrients through the cell wall. Nanomaterials inactivate the proteins, decreasing the membrane permeability and eventually causing the cellular death (Zhang and Chen, 2009).
Silver ions have been used in many kinds of formulations, and recently it was shown that hybrids of AgNPs with amphiphilic hyperbranched macromolecules exhibit effective antimicrobial surface coating. The most important application of silver and AgNPs is in the medical industry, such as topical ointments to prevent infection in burns and open wounds. Newly devised AgNPs-coated wound dressings have been a major breakthrough in the management of wounds or infections. To prevent or reduce infections, a new generation of dressings incorporating antimicrobial agents like silver has been developed. Impregnation of wound dressings impregnated with colloidal silver resulted in a strong decrease of pathogen-specific alterations in infected epithelium. The delivery of silver and AgNPs to infected keratinocytes in a moist healing environment is efficient, fast, and active as compared to wound dressing without silver. Similar results with E. coli were obtained with AgNPs (Mudasir et al., 2013).

Antibacterial activity of AgNPs and its combined effects with antibiotics was assessed by disk diffusion method. Each disk was impregnated with 20 µl of AgNPs, to check the combined effect the disk was impregnated with 10 µl of the antibiotic and 10 µl of AgNPs. AgNPs showed enhancing antibacterial property when used in combination with an antibiotic (Mudasir et al., 2013). In the present study also, combined effect of AgNPs with antibiotics is assessed and the use of AgNPs is found to be useful in most results.

**Antitumor activity:**

The anti-tumor effect of AgNPs and Ag+ was reported (Ahamed et al., 2008; Rahman et al., 2009). In this study, in vitro antitumor activity of the AgNPs was evaluated against HCT -116, MCF-7 and HepG2 cell lines at different concentrations. The cytotoxicity analysis of the AgNPs showed a direct dose-response relationship; cytotoxicity increased at higher concentrations (Tables 5& 6). The result revealed that AgNO$_3$ and AgNPs have potent antitumor activity with IC50 values of 12.4 and 1.2 µg/ml, respectively against HepG2 cells; 14.9 and 1.4 µg/ml, respectively against HCT -116 cells; 3.0 and 2.1 µg/ml, respectively against MCF-7 cells (Fig. 11). The enhanced cytotoxicity of AgNPs may be due to their size which facilitates their subsequent penetration in tumor cells. The cytotoxic effects of AgNPs, probably due to the fact that AgNPs are likely to interact with thiol rich enzymes (Morones et al., 2005), other researchers suggest that AgNPs may interfere with the proper functioning of cellular proteins and induce subsequent changes in cellular chemistry (Rogers et al., 2008); Therefore, it is possible that once penetrated into cells, AgNPs may attack functional proteins of the infected cell and thus causing cell death.
proteins of cells which results in partial unfolding and aggregation of proteins as it is the case in the bovine haemoglobin. Toxicity of silver nanoparticles is concentration-size-shape dependent; in the green process for synthesis of nanoparticles, these factors are affected by chemical compositions of fungal extracts, accordingly this will lead to variability in the biological activities of such extracts (Elechiguerra et al., 2005; Morones et al., 2005; Okafor, 2013).

Table 5. In vitro cytotoxicity effect of AgNO3, Ag nanoparticles and aqueous extract on HCT-116 and MCF-7 cell lines

<table>
<thead>
<tr>
<th>Sample concentration (µg/ml)</th>
<th>Viability% of HCT-116</th>
<th>Viability % of MCF-7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AgNO3</td>
<td>Ag NPs</td>
</tr>
<tr>
<td>100</td>
<td>11.52</td>
<td>1.34</td>
</tr>
<tr>
<td>50</td>
<td>15.86</td>
<td>4.36</td>
</tr>
<tr>
<td>25</td>
<td>34.64</td>
<td>8.91</td>
</tr>
<tr>
<td>12.5</td>
<td>53.67</td>
<td>13.74</td>
</tr>
<tr>
<td>6.25</td>
<td>68.90</td>
<td>28.11</td>
</tr>
<tr>
<td>3.125</td>
<td>74.23</td>
<td>37.25</td>
</tr>
<tr>
<td>1.56</td>
<td>83.62</td>
<td>47.38</td>
</tr>
<tr>
<td>0.78</td>
<td>91.28</td>
<td>59.82</td>
</tr>
<tr>
<td>0.39</td>
<td>96.20</td>
<td>73.34</td>
</tr>
<tr>
<td>0</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>IC 50</td>
<td>14.9</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Table 6. In vitro cytotoxicity effect of AgNO3 and Ag nanoparticles aqueous extract on HepG2 cell line

<table>
<thead>
<tr>
<th>Sample concentration (µg/ml)</th>
<th>Viability % of HepG2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AgNO3</td>
</tr>
<tr>
<td>100</td>
<td>11.43</td>
</tr>
<tr>
<td>50</td>
<td>13.94</td>
</tr>
<tr>
<td>25</td>
<td>24.85</td>
</tr>
<tr>
<td>12.5</td>
<td>49.74</td>
</tr>
<tr>
<td>6.25</td>
<td>71.38</td>
</tr>
<tr>
<td>3.125</td>
<td>80.24</td>
</tr>
<tr>
<td>1.56</td>
<td>87.34</td>
</tr>
<tr>
<td>0.78</td>
<td>94.15</td>
</tr>
<tr>
<td>0.39</td>
<td>98.22</td>
</tr>
<tr>
<td>0</td>
<td>100.00</td>
</tr>
<tr>
<td>IC 50</td>
<td>12.4</td>
</tr>
</tbody>
</table>

CONCLUSION:

Nanoparticles can be produced by physical-chemical methods but it requires involvement of hazardous chemicals and many sophisticated techniques which are not easy. On the other hand, the biosynthesis of nanoparticles by microorganisms is quick, consumes less time, it provides satisfactory biosynthesis of nanoparticles and the whole process is very cheap and effective without the involvement of hazardous chemicals. In the present study, Aspergillus ochraceus (RCMB 036254) was exploited to biosynthesize silver nanoparticles by reducing silver nitrate. These nanoparticles can be produced by physical-chemical methods but it requires involvement of hazardous chemicals and many sophisticated techniques which are not easy. On the other hand, the biosynthesis of nanoparticles by microorganisms is quick, consumes less time, it provides satisfactory biosynthesis of nanoparticles and the whole process is very cheap and effective without the involvement of hazardous chemicals. In the present study, Aspergillus ochraceus (RCMB 036254) was exploited to biosynthesize silver nanoparticles by reducing silver nitrate. These
AgNPs were found to be more active against MRSA when used in combination with antibiotics. It is known that the nanoparticles are so small in size that they can pass through the cell membrane easily. This property is exploited to treat diseases like cancer which is a fatal disease. Chemotherapies which are used to treat cancer have many side effects so there is a need to find some other alternative which could treat it without causing side effects. The mycosynthesized AgNPs showed remarkable anticancer activity against human colon carcinoma, human breast cancer, and human hepatocellular carcinoma cells. With the application point of view, a suitable pharmacological formulation using these nanoparticles, as well as studies on different biological activities in different fields should be strengthened in future studies.

REFERENCES:


I ISSN: 1687-7497 On Line ISSN: 2090 - 0503 http://www.egyseb.org
التكوين الحيوي لدفائق القصة النانوية باستخدام القطرات والتقييم البيولوجي لتلك الدفائق النانوية

هاني محمد مسعود، محمد حسني السيد مراد، مروة مصطفى عبد العزيز

المستقبل الاقليمي للفطرات وتطبيقاتها، جامعة الأزهر، مصر

(برسا) وقد أظهر التجارب أنه من بين ثلاثة عشر مضادًا جيدًا وحيد خمسة مصادر جيدة فقط أعطت زيادة في النشاط ضد الفيروس عند خلطه بالدفائق القصية النانوية، أضاف أيضًا تلك الدفائق القصية بقدرها المنطة للخلايا السرطانية وتعتبر تلك الفترة على تركيز الدفائق القصية وكان التركيز المثلي لصف عدد الخلايا هو 1.4, 2.1, 2, 1.2 مكروجم/ملي ضد كل من خلايا سرطان القولون وسرطان الندى وسرطان الكبد على التوالي، في حين أن التركيز المثلي لشعبة 50% من الخلايا السرطانية هو 39.6 مكروجم/ملي. وقد أوضح تلك الدراسة إمكانية إنتاج دفائق صحة نانوية من قطر أسرجليس أوكريشينز والتي يمكن استخدامها للقضاء على الخلايا السرطانية بتركيزات لتصبح خلايا جيدة الطبية، وأيضًا استخدامها ضد الميكروبات المقاومة للمضادات الحيوية.

المحكمين:
أ.د. إبراهيم كرم الدين، قسم الميكروبيولوجيا، طالب عين شمس.
أ.د. علاء مصطفى أوريد، قسم النبات، صناعة طه.