International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.8, No.4, pp 691-695, 2015

Cytotoxicity of Biologically Synthesized Silver Nanoparticles from Citrus Lemon Against some Cell Lines

Raju Nalvolthula[1], Ramchander Merugu[2], Jahnavi Alwala[1], M.P.Pratap Rudra[1]*

[1]Department of Biochemistry, Osmania University, Hyderabad, India
[2]University College of Science, Mahatma Gandhi University, Nalgonda, India

Abstract: In the present study, silver nanoparticles were synthesized in an ecofriendly method using Citrus lemon and characterized using various characterization techniques. Cytotoxicity of the silver nanoparticles was investigated. Our results indicate that CLNPs prepared from flower extracts of Citrus lemon are active against all cell lines including U937, Colo205, B16F10, HepG2 and HeLa at below 200µg/ml concentration. COLO205 cells are more sensitive for NENPs (IC50: 65.40±2.41) followed by U937 (IC50: 84.17±2.13), HeLa (IC50: 93.27±2.53), HepG2 (IC50: 95.52±4.08) and B16F10 (IC50: 196.5±4.19). The order of sensitivity of human cancer cell lines towards the CLNPs is Colo205> U937 >HeLa >HepG2 >B16F10. The biologically synthesized CLNPs exhibited cytotoxic properties against all the cell lines (<200 µg/ml) in a concentration-dependent manner. The results of the present work are communicated.
Keywords: Silver nanoparticles, cytotoxicity activity, Citrus lemon.

Introduction

Metal nanoparticles of silver have unique properties, such as chemical stability, good electrical conductivity, catalytic and antibacterial activity. Biological synthesis is advantageous compared to chemical synthesis as they are cost effective and ecofriendly .Moreover, they do not involve the use of toxic chemicals [1]. Traditionally Silver nanoparticles and gold nanoparticles were synthesized using citrate salts and ascorbic acid as reducing agents respectively [2, 3] , hence citrus lemon was used for the synthesis if silver nanoparticles. The unique properties are applied in many fields for antimicrobial activity, antitumor activity, cell tagging, cytocompatability, drug delivery. Conventional chemical methods use reducing agents such as ethanol, formaldehyde, hydrazine hydrate, sodium borohydrate and ethylene glycol[4]. Biobased enzymes or phytochemicals are usually responsible for reduction of metal compounds into nanoparticles [5]. In continuation of our earlier work on nanoparticles synthesis [6-12], in this investigation, plant mediated synthesis of silver nanoparticles were carried out using citrus lemon. They were characterized using, Fourier transform infrared spectra, UV-visible spectra and electron microscopy. The synthesized nanoparticles were investigated for their cytotoxicity. The results are presented below and discussed.

Material and Methods

The procedure for synthesis of silver nanoparticles was similar to that of Prathna et al., 2011 [2]. Fresh fruits of lemon were taken, washed, cut, and squeezed and the extract was taken. This extract was then filtered using Whatmann filter paper. The mixture was boiled for 10 minutes and decanted. 10ml of the above prepared extract was taken in 5 separate flasks. 50 mL of aqueous AgNO3 solution (1mM, 2mM, 3mM, 4mM, and 5mM)

was added to the respective flasks drop by drop with continuous stirring for reduction of Ag+ ions at room temperature under dark conditions. 3mL of 8%w/v SDS was added to the above solution. The conical flasks were sealed using cotton plugs and observed for color change. To confirm the reduction of silver ions, the absorption spectra were obtained in the range of 200-700nm with a spectrophotometer (ELICO SL-159 UV-Vis spectrophotometer). The and morphology of nanoparticles were analyzed with a Transmission electron microscope (Philips model CM 200) and scanning electron microscope (Ziess 700 scanning electron microscope) . The purified suspension was oven dried and the powder was subjected to FTIR spectrometry analysis (Perkin Elmer-RX1 Spectrophotometer) in the diffuse reflectance mode at a resolution of $4cm^{-1}$ in KBr pellet. The cell lines U937 (human histiocytic lymphoma), COLO205 (human Colon adenocarcinoma), B16F10 (mouse mealanocarcinoma) HepG2 (hepato cellular carcinoma) and HeLa (human cervix carcinoma) cell lines were obtained from the National Centre for Cellular Sciences (NCCS), Pune, India. Cells were used between passages 10 and 20 and cultured either in RPMI -1640 (U937, COLO205), DMEM (B16F10, HepG2) and MEM (HeLa) media, supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1 mM NaHCO3, 2 mM -glutamine, 100 units/ml penicillin and 100 µg/ml streptomycin. All cell lines were maintained in culture at 37° C in an atmosphere of 5% CO_2. At 85% confluence, cells were harvested using 0.25% trypsin and the cells (2 x 104) were seeded in each well containing 100µl of medium in 96 well plates.Cells were allowed to attach the surface for 24 h prior to nanoparticles exposure. CLNPs were suspended in complete cell culture medium (8mg/ml stock) and diluted to appropriate concentrations (25, 50, 75, 100, 150 and 200µg/ml). The dilutions of CLNPSs were then sonicated using a sonicator bath at room temperature for 15 min at 40W to avoid nanoparticles agglomeration prior to administration to the cells. Selection of 25–200µg/ml concentration of CLNPs was based on a preliminary dose-response study.

Cytotoxicity was measured using the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] assay, according to the method of Mossman (1983) [13]. Briefly, the cells (2 x 104) were seeded in each well containing 100µl of medium in 96 well plates. After overnight incubation at 37 °C in 5% CO2, exactly 100µl of different test concentrations (25µg to 200µg/ml) of CLNPs were added to the cell suspension, which is equivalent to 5 to 40µg per 200µl of assay volume. The viability of cells was assessed after 24h, by adding 10µl of MTT (5 mg/ml) per well and incubated at 37°C for additional three hours. The medium was discarded and the formazan blue, which formed in the cells, was dissolved in 100 µl of DMSO. The intensity of colour formation was measured at 570 nm in a spectrophotometer (Spectra MAX Plus; Molecular Devices; supported by SOFTmax PRO-5.4). The percent inhibition of cell viability was determined with reference to the control values (without test nanoparticles). The data were subjected to linear regression analysis and the regression lines were plotted for the best straight-line fit. The IC50 (inhibition of cell viability) concentrations were calculated using the respective regression equation.

Results and Discussion

Table-1 *In vitro* Cytotoxicity of biologically synthesized CLNPS against U937, Colo-205, B16F10, Hep G2 & Hela cells by MTT assay.

S.NO	Cell line	IC_{50} values of CLNPs (µg/ml)	IC_{50} values of Etoposide (µg/ml)
1	U937	54.14±3.1	5.02±0.62
2	Colo205	44.97±1.25	4.07±0.12
3	B16F10	162.56±3.3	4.12±0.42
4	HepG2	66.77±1.87	3.12±0.26
5	HeLa	149.83±4.1	2.12±0.32

The UV visible spectrum, FTIR spectrum, SEM analysis and TEM images were used to confirm the synthesis of the nanoparticles. The biological activities of CLNPs were prepared from flower extracts of Citrus lemon were evaluated to investigate their anti-proliferative/cytotoxic activities in five different types of human cancer cells including U937 (human histiocytic lymphoma), COLO205 (human Colon adenocarcinoma), B16F10 (mouse mealanocarcinoma) HepG2 (hepato cellular carcinoma) and HeLa (human cervix carcinoma) cells. Our results indicate that CLNPs were prepared from flower extracts of Citrus lemon are active against all

cell lines including U937, Colo205, B16F10, HepG2 and HeLa at below 200µg/ml concentration (Table-1). COLO205 cells are more sensitive (IC50: 65.40±2.41) followed by U937 (IC50: 84.17±2.13), HeLa (IC50: 93.27±2.53), HepG2 (IC50: 95.52±4.08) and B16F10 (IC50: 196.5±4.19). The order of sensitivity of human cancer cell lines towards the CLNPs is Colo205> U937 >HeLa >HepG2 >B16F10. The biologically synthesized CLNPs exhibited cytotoxic properties against all the cell lines (<200 µg/ml) in a concentration-dependent manner. It is evident from the overall results, that the biologically synthesized CLNPs showed the potent anti-proliferative activity against Colo205 cell line, which is less cytotoxic than the positive control, Etoposide (Table 1). Exponentially growing cells were treated with different concentrations of CLNPS for 24h and cell growth inhibition was analyzed through MTT assay. IC_{50} is defined as the concentration, which results in a 50% decrease in cell number as compared with that of the control cultures in the absence of an inhibitor and were calculated using the respective regression analysis. The values represent the mean ± SE of three individual observations. Etoposide is a standard drug molecule employed as positive control.

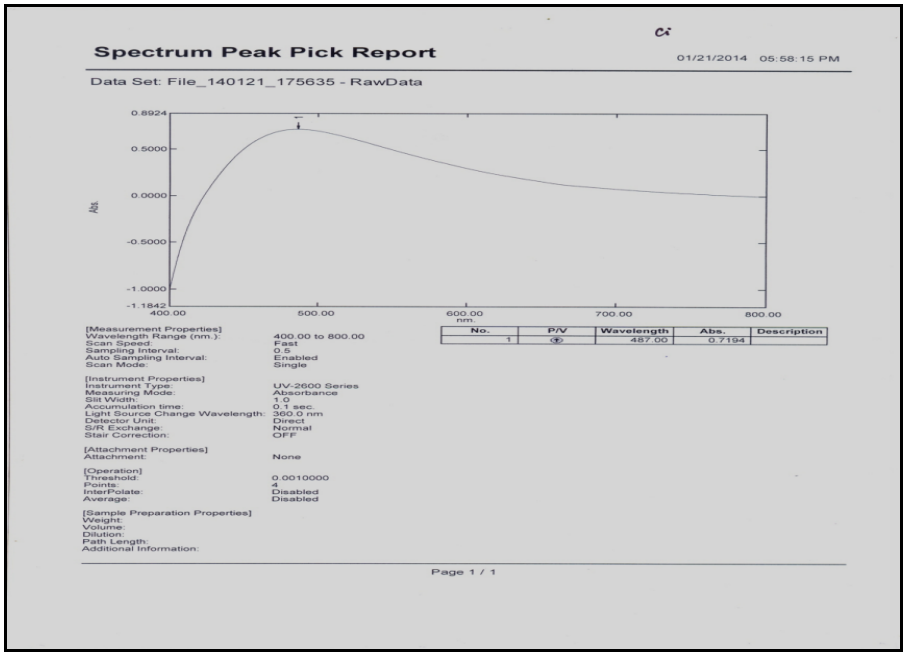

Figure 1: UV-Vis analysis of nanoparticles

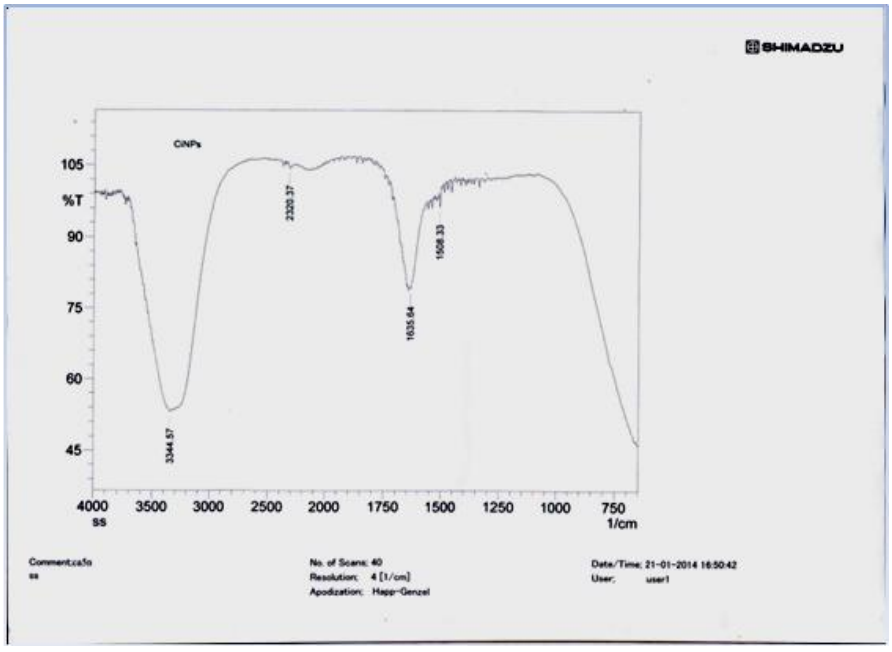

Figure 2: FTIR analysis of nanoparticles

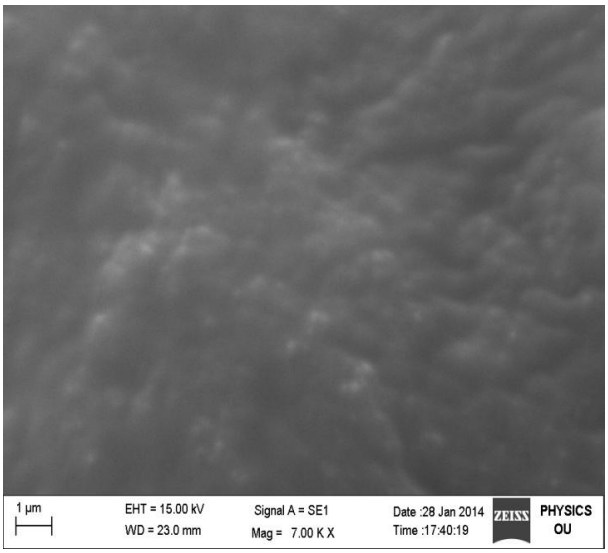

Figure 3: SEM Analysis of nanoparticles

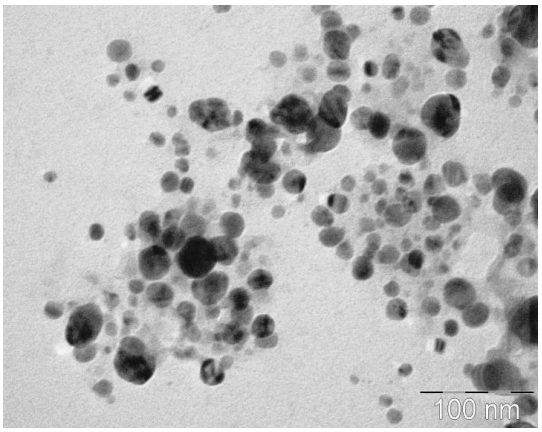

Figure 4: TEM Analysis of nanoparticles

Conclusions

Differential anticancer properties of the biologically synthesized CLNPS against cell lines (U937, COLO205, B16F10, HepG2 and HeLa) may be due to different mechanism of action. These preliminary results indicated that slight modification of methodology for biologically synthesis of CLNPS may yield as prospective anticancer drugs. Based on the present results, it is warranted that these CLNPS to be further evaluated on other cancer cell lines.

References

1. U.K. Parashar, P.S. Saxena and A. Srivastava, Digest J. Nanomater. Biostruct., 4, 159 (2009).
2. D.V. Goia and E. Matijevic, Colloids Surf. A Physicochem. Eng. Asp., 146, 139 (1999)
3. Prathna TC, Chandrasekaran N, Raichur AM, Mukherjee A. (2011). "Biomimetic Synthesis of Silver Nanoparticles by Citrus limon(lemon) Aqueous Extract and Theoretical Prediction of Particle Size". Colloids Surf B Biointerfaces. 82(1): 152-159.

4. P.K. Sahoo, S.S. Kalyan Kamal, T. Jagadeesh Kumar, B. Sreedhar, A.K. Singh & S.K. Srivastava (2009). "Synthesis of Silver Nanoparticles using Facile Wet Chemical Route". Defence Science Journal. 59(4): 447-455
5. Balaprasad Ankamwar, Chinmay Damle, Absar Ahmad, MuraliSastry (2005). "Biosynthesis of Gold and Silver Nanoparticles Using Emblica officinalis Fruit Extract, Their Phase Transfer and Transmetallization in an Organic Solution"
6. Rama Koyyati, Veerababu Nagati, Ramchander Merugu & Prataprudra Manthurpadigya(2014) Antibacterial activity of silver nanoparticles using amaranthus viridis twig extract extract . Int.J.Res.Pharm.sci 5(1): 32-39
7. Rama Koyyati, Veerababu Nagati, Ramchander Merugu & Prataprudra Manthurpadigya (2013) Biological synthesis of silver nanoparticles using Raphanus sativus var. longipinnatus leaf extract and evaluation of their antioxidant and antibacterial activity. International Journal of Medicine and Pharmaceutical Sciences 3(4): 89-100
8. Raju Nalvothula, Veera Babu Nagati, Rama Koyyati, Ramchander Merugu, Pratap Rudra Manthur Padigya.(2014) Biogenic Synthesis of Silver Nanoparticles Using Tectona grandis Leaf Extract and evaluation of their Antibacterial Potential International Journal of ChemTech Research .6(1): 293-298 13.
9. Karunakar Rao kudle, Manisha R Donda, Ramchander merugu,Y.Prashanthi, M.P.Pratap Rudra(2013). Green synthesis of silver nanoparticle using water soluble gum of Sterculia foetida- gum and evaluation of itsantimicrobial activity Int.J.Res.Pharm.sci 4(4):563-568
10. Karunakar Rao Kudle, Manisha R Donda , Y. Prashanthi, Ramchander Merugu, M.P.Pratap Rudra. Synthesis of silver nanoparticles using the medicinal plant Allmania nadiflora and evaluation of its anti microbial activities. Int.J.Res.Pharm.sci 4(4):504-511
11. KR Kudle, MR Donda, MR Kudle, R Merugu, Y Prashanthi, MPP Rudra (2013) . Fruit (epicarp and endocarp) extract mediated synthesis of silver nanoparticles from Sterculia foetida plant and evaluation of their antimicrobial activity Nanoscience and Nanotechnology: An International Journal 2013; 3(3): 56-59
12. Karunakar Rao Kudle, Manisha R. Donda, Ramchander Merugu, Madhukar Rao Kudle, M.P. Pratap Rudra (2013). Microwave assisted green synthesis of silver nanoparticles using Boswellia serrata flower extract and evaluation of their antimicrobial activity. International Research Journal of Pharmacy, DOI: 10.7897/2230-8407. 4 (5).
13. Mosmann T. 1983. Rapid Colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods.65: 55−63.

*****