

Investigation on the Cytotoxicity of Green Synthesis and Characterization of Silver nanoparticles using *Justicia adhatoda Leaves* on human epitheloid carcinoma cells and evaluation of their antibacterial activity

Karunakar Rao Kudle¹

Manisha R Donda¹

Madhukar rao kudle²

Ramchander Merugu³

Y. Prashanthi³

Page

M. P. Pratap Rudra^{1*}

¹Department of Biochemistry, Osmania University, Hyderabad-500007.

²Department of Biochemistry, Kakatiya University, Warangal.A.P, India.

³University College of Science and Informatics, Mahatma Gandhi University, Nalgonda-508254. Abstract:

Green nanotechnology, the science that utilizes the various plant resources for the synthesis of nanoparticles without posing any chemical hazards is proved to be an efficient application in conservation of energy and environment. In the present study, we report biosynthesis of silver nanoparticles using silver nitrate and the leaves extract of the plant Justicia adhatoda which acts as a reducing agent. Formation of nanoparticles was indicated by a color change from pale yellow to reddish brown. Characterization studies were done using UV-Spectrophotometer and FTIR. A peak at 460 nm was obtained, confirming the formation of silver nanoparticles. The Scanning Electron Microscopy and Transmission Electron Microscopy studies revealed that the formed silver nanoparticles were spherical in shape with a smooth morphology and maximum number of particles was within the size range of 11nm-20nm. The antibacterial activity of the synthesized silver nanoparticles was studied against different bacterial species and was found to be highly toxic.

Keywords: Silver nanoparticles, Justicia adhatoda, Green synthesis, FTIR, SEM, TEM, Antibacterial activity, Cytotoxicity.

Corresponding Authors: Email: mpprataprudra@gmail.com

NTRODUCTION:

Nanotechnology is a field of applied science and technology whose control of matter on atomic and molecular scale generally about 1 to 100 nm. Nan particles or nano crystals are made of either metals, semiconductors or oxides with different electrical, optical and chemical properties. They have been used as chemical catalysts and quantum dots. Nana particles are constituted by bounded atoms roughly in the range of 10 to 10⁴. Hence, they cannot be simply treated as free atoms or small gas molecules. On the other hand, they are much smaller than polymers. When the size is too small, particles gain wave character. Hence, the sole particle behaviour, the energy response will be modified. The properties of nanoparticles changes drastically with size. Nanoparticles can be synthesized using physical, chemical and biological methods. .Synthesis using biological sources are rapidly gaining importance owing to their growing success and simplicity. Many biological organisms are known to produce nano materials either intra or extracellularly (1,2,3).

Synthesis of silver and gold and silver nanoparticles have been reported using the plant extracts of Aloevera (4),Tamarind, Emblica officinalis (5,6), Carica papaya (7),Azadirachta indica (8),Jatropha curcas (9),Cuminum cyminum , Stigmaphyllon littorale and ,Boswellia serrata

© 2014 M. P. Pratap Rudra et al, publisher and licensee IYPF. This is an Open Access article which permits unrestricted noncommercial use, provided the original work is properly cited.

(10,11,12) and, Securinega leucopyrus (13), etc. in the present study, we repot the synthesis of silver nanoparticles mediated by the leaves extracts of the medicinal plant Justicia adhatoda. Justicia adhatoda is a herbal plant, also called Vaidyamata singhee belongs to Acanthaceae family and grows wild in abundance all over India. The plant is a source of vitamin C and acts as anti plasmodia, anti diabetic, anti-jaundice, bronchodilator, anti inflammatory and cardio protective agent. The leaves are used for checking postpartum hemorrhage and urinary trouble. The antimicrobial properties of silver nanoparticles were known since ancient times and silver ions are widely used as bactericidal agent. The leaves with such medicinal properties are used for the synthesis of silver nanoparticles which would act as effective application in the field of medicine.

MATERIAL AND METHODS:

Biosynthesis of silver nanoparticles:

Fresh and mature leaves of the plant Justicia adathoda was collected from the Osmania University campus, Hyderabad. The leaves are thoroughly washed with distilled water and cut into small pieces. The plant leaf broth solution was prepared by using 5 gm of washed and cut leaves in a 250 ml Erlenmeyer flask with 50 ml of distilled water and then boiling the mixture for 5 min. the aqueous extract was then filtered by Whitman No 1 filter paper and stored at 4°C. for the synthesis of silver nanoparticles the leaves extract and 1m M silver nitrate solution are added in the ratio 1:10 ratio and kept on a sand bath for 60°C for 10mins. The color of the reaction mixture has changed from pale yellow to reddish brown after incubation.

CHARACTERIZATION STUDIES:

The synthesized silver nanoparticles from the d extract of the plant of Justicia adhatoda were characterized with the help of Elico SL-159 UV Spectrophotometer by continuous scanning from 300nm to 700nm and the extract was used as the reference for the baseline correction. The binding properties behind the formation of silver nanoparticles using extracts of the plant were analyzed by FT-IR. The spectra were recorded in the wave number range of 500-4000cm⁻¹. The analysis was carried using Paragon 500, Perkin Elmer-RX1 spectrophotometer in the diffuse reflectance mode at a resolution of 4cm⁻¹ in KBr pellets. Further, the formed silver nanoparticles were centrifuged at 12,000 rpm for 15min, followed by redispersion of the pellet of silver nanoparticles in 5ml of double distilled water. . The synthesis of silver Nanoparticles image size study by SEM-EDX. The synthesized Nanoparticles morphology were characterized by scanning electron microscope. The elemental analysis of the sample was done using EDX analysis. The size of the nanoparticles was determined using TEM analysis. A drop of the solution was subsequently deposited onto a lacey C film supported on a Cu grid and allowed to evaporate under ambience conditions .The antibacterial assays of silver nanoparticles were done on human pathogens Gram positive and gram negative by disc diffusion method. In this disc diffusion method L.B Agar medium and sterile paper disc containing silver nanoparticles along with standard disc were placed in the Petri plate and incubated at 37° C for 48 hrs and observation was recorded. Ampicilline was used as the positive control.

Covered in Scopus & Embase, Elsevier Int. J. Drug Dev. & Res., January - March 2014, 6 (1): 113-119 © 2014 M. P. Pratap Rudra et al, publisher and licensee IYPF. This is an Open Access article which permits unrestricted noncommercial use, provided the original work is properly cited.

Hela cell line maintenance and growth

Cell line maintenance and growth conditions the cytotoxicity potential of silver nanoparticle was studied against HeLa cell lines (Human epitheloid cervix carcinoma). Cell lines were purchased from NCCS, Pune, India. Hela cell lines were sub cultured and were maintained at 37°C at 5% CO2 in CO2 incubator. Cultures were continuously 24hr observed under every an inverted microscope and to confirm the absence of any bacterial and fungal contaminants.

Evaluation of cytotoxicity activity of the silver nanoparticles by MTT Assay

In present paper in-vitro study of cytotoxicity effect of silver nanoparticle was assessed by MTT (3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay. The process of assay is a colorimetric, non-radioactive assay for measuring cell viability through increased metabolization of tetrazolium salt (14). HelaCell lines were subcultured and 100µl of media (containing 5000cells) were transferred into 96 well plates and incubated for 24 hr. The media was removed and added fresh media 100µl. Synthesized silver nanoparticle was added different at concentration (10-80 µg) and then final volume was made to 100µl with the media and incubated for 4 hr. After incubation media containing drug was removed. 10µl of MTT reagent (5mg/ml in PBS) was added to each well containing media and incubated for 2 hr at 37 °C under an atmosphere of 5% CO2 in incubator until a purple precipitate was observed. Media was removed (Don t disturb cells). 100µl DMSO (MTT solvent) was added to dissolve the purple precipitate (15). Absorbance was read at 562 nm. Percentage cytotoxicity was calculated as for protocol (16) and used for finding the IC50 value of the concentration

required for 50% cell death by synthesis justicia adhatoda leaves silver nanoparticle.

RESULTS AND DISCUSSION:

The silver nanoparticles exhibit yellow brownish color in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles (17). Upon incubation of mixture of the leaves extract with 1mM AgNO₃ solution, the color of the solution changes from pale yellow to dark brown color (Fig. 1). In the FTIR study (Fig 2.), the peaks are more characteristic of eugenols, linalools, and terpenes that are abundant in the leaves of Justicia adhatoda plant leaves. The peaks found at 1635cm⁻¹can be attributed to the C-C in alkene rings and C=C stretch of aromatic rings, respectively, whereas peaks at 1076 cm⁻¹ can be attributed to the ether linkages and C-N stretching vibration of amine. 2344cm⁻¹ cm⁻¹ corresponds to -NH group of amines. 3403 cm⁻¹ shows characteristics of O-H stretching of secondary alcohols. Depending on above observation, it can be assumed that the stabilization is achieved by the phenolic as well as aromatic compounds present in the extract. Studies have confirmed the fact that the carbonyl group form amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly form a layer covering the metal nanoparticles.(Figure. 3) shows SEM images recorded from drop-coated films of the silver nanoparticles synthesized by treating seed powder extract with silver nitrate solution. The formed silver nanoparticles were predominantly spherical in shape. The shape of the nanoparticles plays a key role in optical and electrical properties. With Energy Dispersive Spectroscopy (EDS) analysis, the presence of elemental silver

Covered in Scopus & Embase, Elsevier

Page I

Int. J. Drug Dev. & Res., January - March 2014, 6 (1): 113-119 © 2014 M. P. Pratap Rudra et al, publisher and licensee IYPF. This is an Open Access article which permits unrestricted

noncommercial use, provided the original work is properly cited.

signal was confirmed in the sample (Figure 4and 5) shows TEM images of AgNPs at different magnifications. Images reveal that the formed AgNPs are predominantly spherical in shape with a smooth surface morphology. The figure also shows the histogram of particles size versus number of particles observed on TEM grid. It is clear from the histogram that more number of nanoparticles has the diameter within the range of 11-20 nm and the mean particle size of Ag-NPs is 18 nm. The synthesized silver nanoparticles have shown fairly effective action against different pathogenic bacteria. (Fig: 6, 7) and (table 1).

In this study, we have employed a dose dependent approach to evaluate the cytotoxicity of the nanoparticles on human In-vitro cytotoxicity effect of silver nanoaprticle was studied against HeLa cell lines at different concentration (10, 20, 30, 40, 50, 60, 70, 80µg) (Figure .8). The in-vitro screening of the AgNPs showed potential cytotoxicity activity against the Hela cancer cell lines. The results were shown in Table 2. Complete mortality rate that is 82.15% cell death was observed in 80µg/µl concentration of Ag-NPs. Hence, the inhibitory concentration at 50% (IC50) was fixed at 55µg/µl of Ag-NPs for HeLa cells. At a concentration 50 µg/µl, 59.12% of cytotoxicity and at a concentration 60µg/µl, 41.19% cytotoxicity was recorded and the standard anticancer drug cisplatin (45µg/ml) was also used in this study to confirm and correlate the anticancer activity of Ag-NPs.

Acknowledgements:

The authors are gratefully acknowledged to the University Grants Commission, New Delhi for their financial support in the form of RFMS and Junior Research Fellowship. DBT-OU-ISLARIE -Coordinator

Covered in Scopus & Embase, Elsevier

and CFRD-Director, Osmania University,India for providing all support during the study period.

Authors' contributions:

authors read and approved the final All manuscript. Madhukar Rao Kudle and Prashanthi .Y. Developed the concept and designed experiments. Research guide of this experimental study. Karunakar Rao and Manish R.Donda Performed plant collection, nanoparticles synthesis, and characterization and cell line studies. Instrumental studies and advised on experimental part.



Figure 1: Control (left) and Ag-NPs (right) fabricated from Justicia adhatoda leaves





Int. J. Drug Dev. & Res., January - March 2014, 6 (1): 113-119 © 2014 M. P. Pratap Rudra et al, publisher and licensee IYPF. This is an Open Access article which permits unrestricted noncommercial use, provided the original work is properly cited.



Figure 3: FTIR spectra of silver nanoparticles synthesized by reduction of *Justicia adhatoda* Leaves



Figure: 4. The SEM images of silver Nanoparticles synthesized from *Justicia adhatoda Leaves* Ag-NPs.



Figure 5: TEM micrograph of Silver nanoparticles synthesized using *Justicia adhatoda Leaves* extract









Figure 6: Anti bacterial activity of the synthesized silver nano partials of Justicia Adhatoda extracts

Inhibition zones (mm) of AgNPs synthesized from Justicia Adhatoda leaves Extract against Gram positive and negative bacterial species

buciella species						
Name of microorganism	Leave s Extrac t (10 µl)	Ag- NPs (10µl)	Ag- NPs (20µl)	Ag- NPs (30µl)	Ampicillin e (3µl)	
Bacillus subtilis	5	11	15	20	18	
Klebsiella pneumonia	5	11	14	16	19	
Pseudomonas aeruginosa	5	10	13	17	15	
Pseudomonas putida	5	10	13	16	22	
Escherichia coli	5	11	16	16	23	
Staphylococcu s aurous	5	11	12	14	21	





Fig: 7. Zone of inhibition (mm) of ampicilline (3 μ l/Disc) antibiotics against test strains in absence and in presence of silver nanoparticles (10, 20, 30 μ l/disc).

Covered in Scopus & Embase, Elsevier © 2014 M. P. Pratap Rudra et a

Int. J. Drug Dev. & Res., January - March 2014, 6 (1): 113-119

© 2014 M. P. Pratap Rudra et al, publisher and licensee IYPF. This is an Open Access article which permits unrestricted noncommercial use, provided the original work is properly cited.

SI. No	Concentration of AgNPs (µg/µl)	Percentage of viability		
1	80	17.85±0.52		
2	70	26.64±0.94		
3	60	41.19±0.89		
4	50	59.62±0.52		
5	40	72.21±0.85		
6	30	80.64±0.61		
7	20	93.21±0.87		
8	10	95.46±0.36		
9	Control	100		

Table 2: Percentage of viability of Hela cells add silver nano Particle different concentration effect



Figure 8: Cytotoxicity of Ag-NPs on HeLa cells: increased concentration of Ag-NPs (10-80µg) Xaxis and inhibits the growth of cells up to 100% Yaxis.all the data were expressed in mean ± SD of duplicate experiment

References:

- Gericke, M A. Pinches (2006). Biological synthesis 1) of metal
- 2) of monodisperse gold nanoparticles by a Nanoparticles. Hydrometallurgy 83:132-140.
- Ahmad, A., S. Senapati, M.I. Khan, R. Kumar and 3) M. Sastry. 2003. Extracellular biosynthesis novel Extremophilic actinomycete, Thermomonospora sp. Langmuir, 19: 3550-3553.
- 4) Duran, N.P.D., O.L. Marcato, G.I. Alves, E. Souza, Esposito.2005. Mechanistic aspects of biosynthesis of silver nanaoparticles by several Fusarium oxysporum strains. J. Nanobiotechnol. 3: 8-14.

- Gardea-Torresdey, J.L., J.L. Gomez, J. Peralta-5) Videa, J.G.Parsons, H.E. Troiani and Jose-Yacaman. 2003. Synthesis of Gold Nanotriangles and Silver Nanoparticles using Aloevera plant extract. Langmuir, 13: 13-57.
- Ankamwar, B.D., M. Choudhary and S. Murali. 6) 2005. Gold nanotraigles biologically synthesized using tamarind leaf extract and potential application in vapor sensing. Synth. React. Inorg. Metal-Org. Nanometal Chem. 35:19.
- Ankamwar, B.D., A. Chinmay, A. Absar and S. 7) Murali. 2005. Biosynthesis of Gold and Silver Nanoparticles using Emblica officinalis fruit extract, their phase transfer and transmetallation in an organism solution. J. Nanosci. Nanotechnol. 10: 1665.
- Jain, D., K.D. Hemant, S. Kachhawaha and S.L. 8) Kothari. 2009.Synthesis of plant mediated silver nanoparticles using papaya fruit extract and evaluation of their anti microbial activities. Digest J. Nanomater. and Biostruct. 4: 723.
- Shankar, S.S., A. Rai, A. Ahmad and M. Sastry. 9) 2004. Rapid synthesis of Au, Ag and bimetallic Au core Ag shell nanoparticles using Neem (Azadirachta indica) leaf broth. J. Colloid Interface Sci. 275: 496-502.
- 10) Harekrishna, B., K. B. Dipak, G. Sahoo, P. Sarkar, P. Sankar and A. Misra. 2009. Green synthesis of silver nanoparticles using Jatropha curcas. Colloids and Surfaces A. Physiocochem. Eng. Aspects, 339: 134.
- 11) Karunakar Rao Kudle, Manisha R. Donda, Jahnavi Alwala, Rama Koyyati, Veerababu Nagati, Ramchander Merugu, Y.Prashanthi, M.P.Pratap Rudra. Biofabrication of silver nanoparticles using Cuminum cyminum through microwave irradiation.International Journal of Nanomaterials and Biostructures 2012; 2(4) 65-69.
- 12) Karunakar Rao Kudle, Manisha R. Donda, Ramchander Merugu, Y.Prashanthi, M.P.Pratap Rudra. Microwave assisted green synthesis of silver nanoparticles using Stigmaphyllon littorale leaves, their characterization and anti-microbial

Il Length Original Research Paper

activity. International Journal of Nanomaterials and Biostructures 2013; 3(1): 13-16.

- 13) Karunakar Rao Kudle, Manisha R. Donda, Ramchander Merugu, Madhukar Rao Kudle, M.P. Pratap Rudra. Microwave assisted green synthesis of silver nanoparticles using Boswellia serrata extract and evaluation of their flower antimicrobial activity.International research journal of pharmacy, DOI: 10.7897/2230-8407. 2013, 4 (5).
- 14) Manisha R Donda, Karunakar Rao Kudle, Jahnavi Alwala, Anila Miryala, B Sreedhar and MP Pratap Rudra. Synthesis of silver nanoparticles using extracts of Securinega leucopyrus And evaluation of its antibacterial activity. INT J CURR SCI 2013, 7: E 1-8, ISSN 2250-1770
- 15) Moshmann.T, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983 Dec 16; 65(1-2):55-63.
- 16) Renugadevi .K, R.VenusAswini: Microwave irradiation assisted synthesis of silver nanoparticle using Azadirachtaindicaleaf extract as a reducing agent and its invitro evaluation of its antibacterial and anticancer activity. International Journal of Nanomaterials and Biostructures 2012; 2(2): 5-10.
- 17) Murugaraj Jeyaraj, et., al. An investigation on the cytotoxicity and caspase-mediated apoptotic effect biologically synthesized of silver nanoparticles using Podophyllumhexandrum on human cervical carcinoma cells. Colloids and Surfaces B: Biointerfaces 102 (2013) 708-717.
- 18) Mulvaney, Ρ., 1996, *Surface* plasmon spectroscopy of nanosized metal particles,"Langmuir 12, pp. 788-800.

Article History: -----

Date of Submission: 14-11-2013 Date of Acceptance: 14-12-2013 Conflict of Interest: NIL Source of Support: NONE





Covered in Scopus & Embase, Elsevier © 2014 M. P. Pratap Rudra et al, publisher and licensee IYPF. This is an Open Access article which permits unrestricted noncommercial use, provided the original work is properly cited.

Int. J. Drug Dev. & Res., January - March 2014, 6 (1): 113-119

Page