GREEN BIOSYNTHESIS OF SILVER NANOPARTICLES CONJUGATED TO GEFITINIB AS DELIVERY VEHICLE

[1]SAMRA KHALID, [2]RUMEZA HANIF

[1,2]Department of Atta-ur-Rahman School of Applied Biosciences (ASAB)/Healthcare Biotechnology, National University of Sciences and Technology, Islamabad, Pakistan
E-mail: [1]samra.12.tfrphd.90066tp@asab.nust.edu.pk

Abstract- Green synthesized silver nanoparticles (AgNPs) were extracted from Aerva javanica plant using eco-friendly green chemistry approach. Anti-cancer drug gefitinib conjugated to these spherical shaped with an average size of 5.7nm AgNPs. Gefitinib-conjugated AgNPs were characterized by ultraviolet–visible spectroscopy, fourier transform-infrared (FT-IR) and scanning transmission electron microscopy (STEM) analysis. These results were confirmed the conjugation of gefitinib with AgNPs at 490nm wavelength. The effect of acidic pH 4.5 showed the agglomerate formation of gefitinib in significant amount in a shorter period which delivers drugs effectively in a tumor cells. The cell viability was analysed in human breast cancer cell line (MCF-7) determined that gefitinib-AgNPs were 50% more effective than gefitinib alone. It was concluded that the gefitinib conjugated AgNPs are used as effective delivery vehicle which are less toxic in nature.

Keywords- Silver nanoparticles, Aerva javanica, Gefitinib, Breast cancer.

I. INTRODUCTION

Nanotechnology confers the nano-scale architecture and design of materials [1] as nano-materials have increased surface area which enhances their optical, physical and chemical properties [2]. Nano-material preparations used in biomedical applications as targeted drug delivery, cancer treatment, molecular imaging, endocrine resistant therapy, genomic DNA analysis, photothermal therapy, antibacterial agents and as biosensors [3, 4]. Silver nanoparticles (AgNPs) are significant class of metallic nanoparticles and have been extensively used in industry, pharmaceuticals, as disinfectants [5]. They have been employed in biomedical applications owing to excellent biocompatibility and size dependent properties [6]. Biological synthesis of AgNPs has reduced cytotoxicity due to the presence of capping agents and hence can be used as drug delivery vehicle because of their biocompatibility and biofunctionality [7]. In current study, we were reported the synthesis of non-toxic quantum sized fluorescent AgNPs from Aerva javanica [8].

Aerva javanica has been known for its medicinal properties. It has been used in conventional medicine as antifungal, antibacterial agent [8]. Anthelminthic properties of Aerva javanica has also been reported [9]. Extensive use of Aerva javanica (common name Hathi bovei) in skin treatment because of its antioxidant properties [8]. These antioxidant properties of Aerva javanica are due to presence of alkaloids, flavonoids and other biochemical compounds present in Aerva javanica Alkaloids isolated from Aerva sp. exhibit characteristic green fluorescence [10-12]. Phenols exhibit good antioxidant and reducing properties [13] which is required for synthesis of AgNPs [14]. As these phenol groups are oxidized quinoid compounds are produced which have the ability to bind to the surface of nanoparticles responsible for capping and stability of nanoparticles [15]. Hence, the synthesized nanoparticle was capped with antioxidant capping agent thereby reducing the cytotoxicity of these nanoparticles. Combination of fluorescent and antioxidant properties of AgNPs may provide an efficient alternative for cytotoxic quantum dots for their biomedical applications including drug delivery and bioimaging [16].

Chemically synthesized AgNPs have been shown cytotoxic effects because of generation of reactive oxygen species (ROS) [17]. Effects of AgNPs have been seen on various cell lines including Huh7 cell lines, MCF-7, HepG2, A549 cell lines [18-21]. The exact mechanism depends upon the size and capping agents of AgNPs but chemically synthesized AgNPs reduced the glutathione (GSH) levels and promotes the production of ROS [22]. GSH is an important ROS scavenger; hence play an important role in minimizing oxidative stress by binding to ROS [23]. Hsin et al has shown that AgNP mediated oxidative stress is because of inhibition of GSH synthesizing enzyme thereby increasing oxidative stress within the cell. However, superoxide dismutase and catalases are also inhibited by AgNPs [24]. Cell membrane undergoes lipid peroxidation and DNA and mitochondrial damage occurs as a result of ROS generation. Cytochrome C released from mitochondria causes activation of caspase 9 and caspase 3. This release is due to down regulation of Bcl-2 and up regulation of Bax leading to cellular apoptosis which is caused by AgNPs [24]. Biosynthesized nanoparticles, however, do not exhibit cytotoxic properties because of surface capping which potentially reduces their toxicity [25].

Chemotherapy is in practice for many years and has preferentially leaded to a significant reduction in mortality among patients with breast cancer [26]. However, there is an evidence of multidrug

resistances towards this therapy [26]. To overcome this limitation one of the most promising strategy is the nano-particle delivery of anti-cancerous drugs [27]. Gefitinib also known as IRESSA [4-(3-chloro-4-fluoroanilino)-7-methoxy-6-(3-morpho-linopropoxy)-quinazoline] is a selective inhibitor that exhibit strong anti-tumor efficacy in multiple cancers such as adenocarcinoma [28]. In combination therapy of lung cancer treatment, gefitinib become resistance towards this therapy due to the induction of insulin like growth factors such as IGF-1R [29]. In the present study we were targeted nano-material based drug delivery by conjugating gefitinib to green synthesized AgNPs which significantly enhanced antitumor efficacy for the treatment of breast carcinoma cells.

II. EXPERIMENTAL METHODS

2.1. Synthesis of silver nanoparticles (AgNPs)

AgNPs were synthesized using the chemical reduction method as described by khan [8]. 0.5 gm of Aerva javanica [30] plant extract was added in 1mM solution of AgNO3 (Sigma Aldrich, UK) placed at 200rpm in a rotary shaker (Hauppauge, USA).The reaction time was completed in 6-8 hrs. After reaction, the colour of reaction mixture was changed from yellow to brown indicates that the synthesis of AgNPs. 50 gm of synthesized AgNP solution was mixed with 50% (w/w) of poly ethylene glycol for capping of nano-particles. Ultraviolet–visible (UV-vis) spectrum of nano-silver solution was recorded at 300 to 700 cm-1 on UV Spectrophotometer (Model UVD-2950; USA).

2.2. Conjugation of gifitinib with AgNPs

The gifitinib (Iressa; AstraZeneca, USA) were solubilized using different concentrations ranging from 1 to 50 μgmL−1 in 1% ethanol. The construction of standard curve based on absorbance value was measured at 304nm. 10μgmL−1 drug solution was added drop by drop to the AgNP solution. The formation of gefitinib-conjugated AgNPs was obtained after continuous stirring at 150 rpm for 2hr at room temperature. The prepared solutions were centrifuged at 14,000 × g for 1 hr and pellets were subjected to STEM and FT-IR analyses to confirm the conjugation.

2.3. Characterization of gefitinib-conjugated AgNPs

Effect of drug-conjugated AgNPs was analyzed on 1N HCl and 1 N NaOH pH in phosphate buffer saline solution at 37°C. Drug-conjugated AgNPs was initially measured using UV-Vis spectrophotometer (Model UVD-2950; USA) at 350 to 700 nm. The transmission spectrum was recorded on fourier transform-infrared (FT-IR) spectrometer (Perkin-Elmer Spectrum, USA) by scanning wavenumber range of 600-4000cm-1. One of efficient technique is scanning transmission electron microscopy (STEM) was used to determine the surface morphology by using STEM detector at 10kV (Nova Nano-SEM450, USA).

2.4. Cell Viability Assay

The cell viability of AgNPs and gefitinib-AgNPs were assessed against MCF-7 cells, obtained from the American Type Culture Collection (ATCC, Manassas, VA). 1×105 cells were grown in 96-well microtitre plate (Becton Dickinson, USA) for 24hrs and treated with different concentrations (10-40 μg/ml) for 48hrs. Later, the cells were incubated with 250μg ml−1 MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma-Aldrich, Biotechnology, USA) for 4hrs at 37°C. The cultured media was replaced with 100μL of dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Biotechnology, USA) after incubation. The absorbance was recorded at a wavelength of 570nm in a microplate reader (Techno Service, AMP Platos R496, Egypt).

2.5. Statistical analysis

Statistical differences of cell viability between groups were performed by un-paired ‘t’ test ($p < 0.05$) using the GraphPad PRISM statistic software (version 7.0) (GraphPad Software, La Jolla, California USA).

III. RESULTS AND DISCUSSION

3.1. Synthesis and conjugation of AgNPs with gefitinib

Medicinal plants have been reported to play an important role in the treatment of various malignancies and Aerva javanica has been known for its antimicrobial, antiulcer, anthelmintic, hypoglycaemic, analgesic and antioxidant properties [8-10]. The cytotoxicity of AgNPs depends on morphology including size, shape, surface chemistry and concentrations of nano-scale AgNPs [31]. Surface capping of AgNPs using polyethylene glycol (PEG) reduces the cytotoxicity [32]. In the present study we used A. javanica sp. strain from our laboratory and characterize the synthesized AgNPs using non-toxic green chemistry approach that is cost effective and environment friendly [8]. These characterized particles were conjugated with gefitinib to synthesize the gifitinib-AgNP.

3.2. Characterization of gefitinib Conjugated AgNPs

Gefitinib-AgNPs was initially confirmed by UV–vis spectrum as shown in Fig. 1. It was showed that wavelength of conjugated AgNPs was 30 fold high (490nm) then the without drug conjugated particles which was well-substantiated by the FT-IR and STEM results. Gefitinib-AgNPs exhibited prominent bands at wavenumber of 3719cm−1, 3040cm−1, 2061cm−1, 1258cm−1 and 723cm−1 matched with the functional groups of AgNPs 3702cm−1, 2862cm−1, 1079cm−1 and 664cm−1 shown in Fig. 2. Presence of hydrophilic group (OH) confirmed by FTIR analysis on the surface of AgNPs synthesized during this study indicated their stability in water because hydrogen bonding with hydrogen atoms of

water molecules. Presence of this group is important as it helps nanoparticles to easily pass out from glomerular filtrate excreted out from kidneys which otherwise may be retained in kidney and can cause damage to it. [33]. Due to their nontoxic nature they can be efficiently employed in drug delivery. Size dependent endocytosis of nanoparticles plays an important role in drug delivery as they can help drug to gain entry into the cells. Clathrin, caveolin, and dynamin mediated endocytosis have been reported along with macro-pinocytosis have been reported to be possible mechanisms where dynamin and clatherin mediated endocytosis occur in case of bigger particles size, particles with small size are endocytosed through caveolin [34]. Particles with size less than 10 nm can be efficiently diffused into the cells and hence into nucleus where they can cause increased toxicity so they should be capped by nontoxic molecules to prevent their toxicity on cells. In line with this our results of STEM analysis showed that the average size of AgNPs is 5.7nm shown in Fig. 3. Effect of pH (4.5 and 7.5) was assessed on gefitinib-AgNPs solution. It was confirmed that high acidic pH 4.5 showed 490nm wavelength similar results observed through FTIR analysis. This causes the agglomerate formation of gefitinib in significant amount in a shorter period which delivers drugs effectively in a tumor cells. Best results were shown at pH of 4.5, with narrow size distribution of gefitinib-conjugated AgNPs as shown in Fig.4.

3.3. Cell Viability

AgNPs have unique application in drug delivery strategies which possess low toxicity, high stability and increased surface area [35]. Previous study reported that anti-cancerous drugs (doxorubicin and alendronate) conjugated AgNPs used as effective delivery vehicle in tumor cells [36]. The plant derived AgNPs were synthesized by green chemistry approach and used for drug delivery [37]. In this study, cell viability was determined in MCF-7 cell line with the treatment of different concentration of gefitinib and gefitinib-AgNPs as compared to the control (shown in Fig.4). Gefitinib-AgNPs showed 50% significant reduction in cell viability of MCF-7 compared to gifitinib alone. This observation suggested that conjugated gefitinib-AgNPs could deliver gefitinib effectively into the breast cancer cells (MCF-7) and caused apoptosis of cancerous cells.

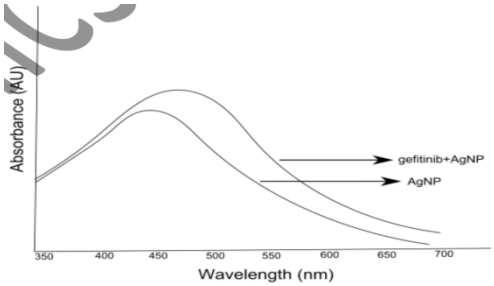

Fig.1. Ultraviolet–visible absorption spectra of AgNPs conjugated with gefitinib.

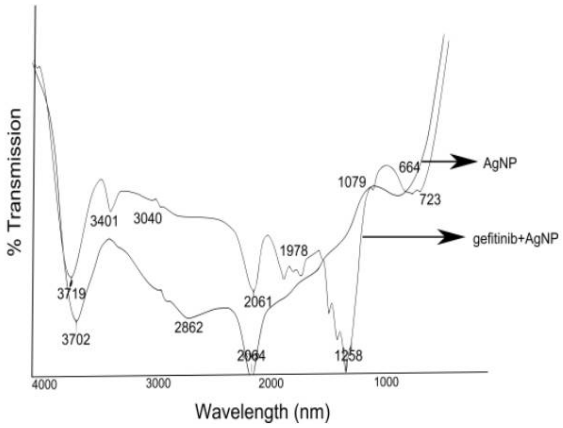

Fig.2. Fourier transform-infrared (FT-IR) spectroscopy of AgNPs conjugated with gefitinib.

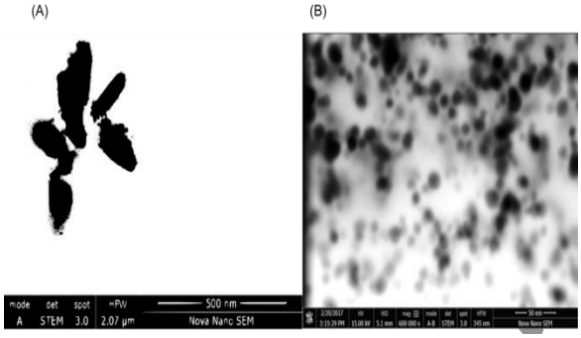

Fig.3. Scanning transmission electron microscopy (STEM) images (A) gefitinib-AgNPs (500nm) and (B) AgNP (50nm) dispersed on carbon-coated copper grid.

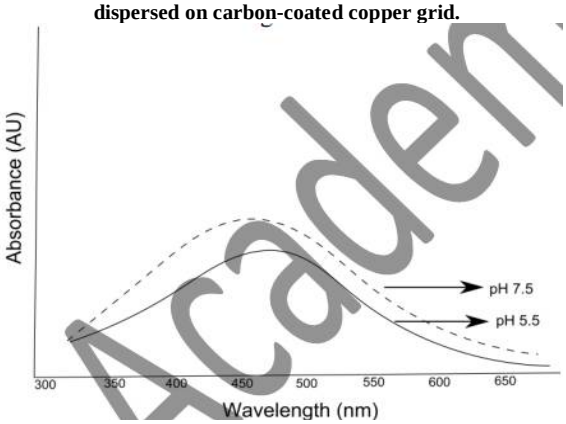

Fig.4. pH dependent absorption spectra of gefitinib-AgNPs

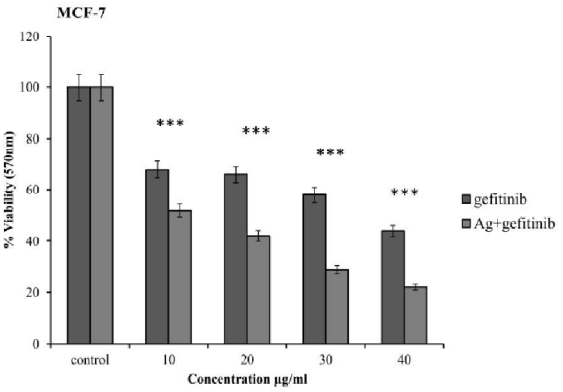

Fig.5. Cell viability of MCF-7 cell line treated with different concentrations of gefitinib and AgNPs+gefitinib measured by using MTT assay. All the experiments were repeated thrice and the data were analyzed by unpaired 't' test; $p < 0.05$ as compared to control group.

CONCLUSIONS

We conclude the following findings in our current study;

1) Biosynthesis of quantum sized fluorescent AgNPs which can be potential quantum dots.

2) Their narrow size distribution range and cytotoxic profile analysis indicates their uniform size dependent properties and their non-toxic nature.

3) Non-toxic AgNPs conjugated with gefitinib in eco-friendly environment and were further characterized.

4) The inhibitory effect of gefitinib conjugated AgNPs against breast carcinoma cells were exhibited as effective drug delivery vehicle.

5) It was concluded that AgNPs has high stability and biocompatibility indicating their potential application in Nanomedicine.

ACKNOWLEDGMENTS

I am grateful to National University of Science and technology of Pakistan for the financial support of this project. I also thankful to Dr. Husnain Janjua from Atta-ur-Rahman School of Applied Biosciences, National University of Sciences and Technology, who provides me research lab facilities. I am grateful to Uzair Hashmi from Atta-ur-Rahman School of Applied Biosciences, National University of Sciences and Technology, Islamabad, Pakistan for providing me synthesized silver nano-particles.

REFERENCES

[1] Roco, M.C., National nanotechnology initiative-past, present, future. Handbook on nanoscience, engineering and technology. Ed. Goddard, WA et al. CRC, Taylor and Francis, Boca Raton and London.—2007.—P, 2007: p. 3.1-3.26.
[2] Gatoo, M.A., et al., Physicochemical properties of nanomaterials: implication in associated toxic manifestations. BioMed research international, 2014. 2014.
[3] Alivisatos, P., The use of nanocrystals in biological detection. Nature biotechnology, 2004. 22(1): p. 47-52.
[4] Salata, O.V., Applications of nanoparticles in biology and medicine. Journal of nanobiotechnology, 2004. 2(1): p. 3.
[5] Deepak, V., et al., An insight into the bacterial biogenesis of silver nanoparticles, industrial production and scale-up, in Metal nanoparticles in microbiology. 2011, Springer. p. 17-35.
[6] Gurunathan, S., et al., Antiangiogenic properties of silver nanoparticles. Biomaterials, 2009. 30(31): p. 6341-6350.
[7] Mansoori, G.A., Principles of nanotechnology: molecular-based study of condensed matter in small systems. 2005: World Scientific Publishing Co Inc.
[8] Khan, A.W., et al., Phytochemical analysis and enzyme inhibition assay of Aerva javanica for ulcer. Chemistry Central Journal, 2012. 6(1): p. 76.
[9] Srinivas, P. and S.R. Reddy, Screening for antibacterial principle and activity of Aerva javanica (Burm. f) Juss. ex Schult. Asian Pacific Journal of Tropical Biomedicine, 2012. 2(2): p. S838-S845.
[10] Chawla, P., et al., A review of chemistry and biological activities of the genus Aerva–a desert plant. Acta Pol Pharm, 2012. 69: p. 171-7.
[11] Saleh, N.A., R.M. Mansour, and K.R. Markham, An acylated isorhamnetin glycoside from Aerva javanica. Phytochemistry, 1990. 29(4): p. 1344-1345.
[12] Goyal, M., et al., Aerva lanata: A review on phytochemistry and pharmacological aspects. Pharmacognosy reviews, 2011. 5(10): p. 195.
[13] Pietta, P.-G., Flavonoids as antioxidants. Journal of natural products, 2000. 63(7): p. 1035-1042.
[14] Martinez-Castanon, G., et al., Synthesis and antibacterial activity of silver nanoparticles with different sizes. Journal of Nanoparticle Research, 2008. 10(8): p. 1343-1348.
[15] Wang, W., et al., One-step synthesis of biocompatible gold nanoparticles using gallic acid in the presence of poly-(N-vinyl-2-pyrrolidone). Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2007. 301(1): p. 73-79.
[16] Selvan, S.T., T.T. Tan, and J.Y. Ying, Robust, Non-Cytotoxic, Silica-Coated CdSe Quantum Dots with Efficient Photoluminescence. Advanced Materials, 2005. 17(13): p. 1620-1625.
[17] Martindale, J.L. and N.J. Holbrook, Cellular response to oxidative stress: signaling for suicide and survival. Journal of cellular physiology, 2002. 192(1): p. 1-15.
[18] Christen, V. and K. Fent, Silica nanoparticles and silver-doped silica nanoparticles induce endoplasmatic reticulum stress response and alter cytochrome P4501A activity. Chemosphere, 2012. 87(4): p. 423-434.
[19] Vivek, R., et al., Green biosynthesis of silver nanoparticles from Annona squamosa leaf extract and its in vitro cytotoxic effect on MCF-7 cells. Process Biochemistry, 2012. 47(12): p. 2405-2410.
[20] Kawata, K., M. Osawa, and S. Okabe, In vitro toxicity of silver nanoparticles at noncytotoxic doses to HepG2 human hepatoma cells. Environmental science
[21] & technology, 2009. 43(15): p. 6046-6051.
[22] Foldbjerg, R., D.A. Dang, and H. Autrup, Cytotoxicity and genotoxicity of silver nanoparticles in the human lung cancer cell line, A549. Archives of toxicology, 2011. 85(7): p. 743-750.
[23] Dewanjee, S., et al., Effective control of type 2 diabetes through antioxidant defense by edible fruits of Diospyros peregrina. Evidence-Based Complementary and Alternative Medicine, 2011. 2011.
[24] Anderson, M.F., et al., Glutathione monoethyl ester provides neuroprotection in a rat model of stroke. Neuroscience letters, 2004. 354(2): p. 163-165.
[25] Hsin, Y.-H., et al., The apoptotic effect of nanosilver is mediated by a ROS-and JNK-dependent mechanism involving the mitochondrial pathway in NIH3T3 cells. Toxicology letters, 2008. 179(3): p. 130-139.
[26] Abdel-Aziz, M.S., et al., Antioxidant and antibacterial activity of silver nanoparticles biosynthesized using Chenopodium murale leaf extract. Journal of saudi chemical society, 2014. 18(4): p. 356-363.
[27] Martin, H.L., L. Smith, and D.C. Tomlinson, Multidrug-resistant breast cancer: current perspectives. Breast Cancer: Targets and Therapy, 2014. 6: p. 1-13.
[28] Yuan, Y., et al., Nanoparticle delivery of anticancer drugs overcomes multidrug resistance in breast cancer. Drug delivery, 2016. 23(9): p. 3350-3357.
[29] Noro, R., et al., Gefitinib (IRESSA) sensitive lung cancer cell lines show phosphorylation of Akt without ligand stimulation. BMC cancer, 2006. 6(1): p. 277.
[30] Ge, X., et al., Induced IGF-1R activation contributes to gefitinib resistance following combined treatment with paclitaxel, cisplatin and gefitinib in A549 lung cancer cells. Oncology reports, 2014. 32(4): p. 1401-1408.
[31] Qureshi, R., G.R. Bhatti, and R.A. Memon, Ethnomedicinal uses of herbs from northern part of Nara desert, Pakistan. Pak J Bot, 2010. 42(2): p. 839-51.

[32] Lovrić, J., et al., Differences in subcellular distribution and toxicity of green and red emitting CdTe quantum dots. Journal of Molecular Medicine, 2005. 83(5): p. 377-385.
[33] Jamieson, T., et al., Biological applications of quantum dots. Biomaterials, 2007. 28(31): p. 4717-4732.
[34] Zhang, Y., et al., Perturbation of physiological systems by nanoparticles. Chemical Society Reviews, 2014. 43(10): p. 3762-3809.
[35] Iversen, T.G., N. Frerker, and K. Sandvig, Uptake of ricinB-quantum dot nanoparticles by a macropinocytosis-like mechanism. Journal of nanobiotechnology, 2012. 10(1): p. 33.
[36] Gupta, D.K., A.N. Chandu, and M. Chouhan, SILVER NANOPARTICLES AS DRUG DELIVERY SYSTEMS. International Journal of Pharmacy & Life Sciences, 2016. 7(9).
[37] Benyettou, F., et al., Synthesis of silver nanoparticles for the dual delivery of doxorubicin and alendronate to cancer cells. Journal of Materials Chemistry B, 2015. 3(36): p. 7237-7245.
[38] Ponarulselvam, S., et al., Synthesis of silver nanoparticles using leaves of Catharanthus roseus Linn. G. Don and their antiplasmodial activities. Asian Pacific Journal of Tropical Biomedicine, 2012. 2(7): p. 574-580.

★★★