Original Research Article Biotechnology

International Journal of Pharma and Bio Sciences

ISSN
0975-6299

ANTI PROLIFERATIVE EFFECT OF SILVER NANOPARTICLES SYNTHESIZED USING HUMAN SERUM ALBUMIN ON MCF-7 CELL LINE.

V. PRADEEPA[1*], M. SUJATHA[2] and T. SRIRAM [2]

[1]Department of Biotechnology, K.S.G College of Arts and Science, Coimbatore, Tamil Nadu, India
[2]Department of Biotechnology, Sri Kaliswari College of Arts and Science, Sivakasi, Tamil Nadu, India

ABSTRACT

A simple and convenient method is synthesis of silver Nanoparticles (AgNps) in a foam matrix using the protein Human serum albumin (HSA) is reported. HAS is an excellent foaming agent and by virtue of its zwitter ionic character at the protein isoelectric point, may be used to bind to cationic silver (Ag+) ions in the foam. The metal ions in the foam are thereafter reduced in situ to yield silver Nanoparticles. The HAS molecules coat and stabilize the Nanoparticles thus eliminating the necessity of employing an additional stabilizing agent in the experimental procedure. The synthesized Nanoparticles where characterized by UV-visible spectroscopy, X-ray diffraction (XRD), Scanning electron microcopy (SEM), energy-dispersive X-ray spectrometry (EDAX) and Fourier-transform infrared spectroscopy (FTI) with silver Nanoparticles of 20-30nm were obtained *invitro* cytotoxic studies revealed that the silver Nanoparticles shows Significant growth inhibition in dose dependent manner.

KEYWORDS: *Silver Nanoparticles, Human Serum Albumin, MCF-7, Cancer, MTT Assay, FTIR*

V. PRADEEPA

**Department of Biotechnology, K.S.G College of Arts and Science,
Coimbatore, Tamil Nadu, India**

*Corresponding Author

Received on: 17-12-2016
Revised and Accepted on 13-02-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.2.b126- 133

INTRODUCTION

Nanotechnology involves the tailoring of materials at atomic level to attain unique properties, which can be suitably manipulated for the desired applications. Nanotechnology is currently employed as a tool to explore the darkest avenues of medical sciences in several ways like imaging, sensing, targeted drug delivery, and gene delivery systems and artificial implants[1]. Biosynthesis of Nanoparticles as an emerging highlight of the intersection of nanotechnology has received increased attention due to growing need to develop environmentally benign technologies in material synthesis[2].The main two approaches are used in nanotechnology. In the "bottom- up" approach, materials and devices are built from molecular components. In the "top- down" approach, Nano objects are obtained from larger entities without atomic level modification[3]. Nanoparticulate carrier system permits entrapment /encapsulation of therapeutics without modification, as it requisite for polymer drug conjugates. Both metallic and polymeric Nanoparticles are used to encapsulate drugs within the solid core. The use of metals can yield multifunctional nanoparticles whereby both therapeutic delivery and imaging are facilitated[4]. Metallic Nanoparticles have fascinated scientists for over a century and are now heavily utilized in biomedical sciences and engineering[5].Silver Nanoparticles (AgNps) have emerged as an arch product from the field of nanotechnology. Silver has gained interest over the years because of distinctive properties, such as good conductivity, chemical stability, catalytic and antibacterial activity[6]. Silver NPs have become efficient vehicles to store and deliver medicines. They are used for the controlled release of drugs, and albumin-drug complexes, where NPs acts as a carrier of drugs and liberate them on a selective basis, at the right speed and in the intended environment within organism. They can be used as magic "bullets" that go directly to cells of a particular tissue[7]. The biological method of synthesis of Nanoparticles have proved to be a better method than the chemical methods due to the large amount of capital involved in production and it involves an energy intensive process. The use of hazardous chemicals and generation of huge amount by products eliminates the method from being an eco-friendly one[8]. The endogenous HSA serves as a suitable material for Nanoparticles formation as albumin is naturally found in the blood and is thus easily degraded, nontoxic, and non immunogenic[9]. Albumin is an acidic protein and remains stable between pH range and temperatures up to 60° C. albumin-based Nanoparticles delivery systems are easily accumulated in tumor tissue due to the enhanced permeability and retention (EPR) effect[10, 11]. The vasculature in an active tumor is different from the vessels found in normal tissue[12]. Based on the above findings, we have synthesized HAS-capped silver Nanoparticles at room temperature by chemical method. In our Experiment HAS is used as template to synthesize the Nanoparticles. These Nanoparticles where characterized by UV-visible spectroscopy, X-ray diffraction (XRD), Scanning electron microcopy (SEM), energy-dispersive X-ray spectrometry (EDAX) and Fourier-transform infrared spectroscopy (FTI) and further screened for *invitro* cytotoxic studies.

MATERIALS AND METHODS

$AgNO_3$ (99.98%) was used as a silver precursor, and was provided by Merk (Darmstadt, Germany). HNO_3 (70%), Human Serum Albumin (HSA) and HCL (37%) were obtained from Sigma-Aldrich (St Lois, MO). The human breast cancer cell line (MCF7) was procured from National centre for Cell Science (NCCS, Pune). All the reagents in this effort were analytical grade and were used as received without further purification. All solution were freshly prepared using double distilled water and kept in a dark to avoid any photochemical reactions. All glass wares used in experimental procedures were cleaned in a fresh solution of HNO_3/HCl (3:1, v/v), washed thoroughly with double distilled water and dried before use

Synthesis of Silver Nanoparticles

Silver Nano particles were prepared by wet chemical synthesis. An albumin solution was prepared dissolving 0.1gms of HSA in 30ml of deionized water. The AgNO3 solution was prepared dissolving 0.05gms of AgNO3 in 10 ml of deionized water. In the HSA solution, AgNO3 solution was added while stirring on a magnetic stirrer and after 5 minutes, ammonium hydroxide was added drop wise to maintain the pH. It was stirred further for ~15 minutes, and to let the solution settle down for 24 hours. The silver nano particles thus formed were isolated by filtration and washed with a minimum of amount of ethanol.

Characterization of NPs

The prepared Ag-NPs were characterized by UV-visible spectroscopy, X-ray diffraction (XRD), Scanning electron microcopy (FESEM), energy-dispersive X-ray spectrometry (EDAX) and Fourier-transform infrared spectroscopy (FTIR). The UV visible spectra were recorded over the 300-800 nm range with a UV-1650 PC UV-visible spectrophotometer[13] (Shimadzu, Osaka, Japan). The structures of silver Nanoparticles produced were examined by XRD (XRD-6000; Shimadzu). The XRD patterns were recorded at a scan speed of 4°/ minute. SEM was performed using a Philips XL-30 instrument (Philips, Eindhoven, Netherlands) to study the morphology of Ag-NPs. The EDAX was carried out on a DX-700HS spectrometer. Meanwhile, the FT-IR spectra were recorded over the range of 400-4000cm^{-1}using an FT-IR series 100, 1650 PerkinElmer spectrophotometer[13] (Los Angeles, CA)

In vitro cytotoxic activity against breast cancer cell line

Human breast cancer cells MCF- 7 were purchased from National Centre for Cell Science (NCCS), Pune, India. Cells were cultured in standard RPMI- 1640 medium containing 2mM glutamine supplemented with 12% (v/v) fetal bovine serum (heat – inactivated FBS) and antibiotics (100 U penicillin/ ml and 100 µg streptomycin/ml) at 37° C and 95 % air + 5 % CO_2 in a humidified incubator. Cells were maintained in 25 or 75 cm^2 flasks and sub cultured every 3 - 4 days.

MTT assay

MCF-7 cells were purchased from National Centre for Cell Science (NCCS), Pune and maintained in DMEM

and McCoys.5a medium, supplemented with non-essential amino acids. Cells were cultured at 37^0 C in a humidified atmosphere containing 5% CO_2 in a CO_2 incubator. Cells were cultured and ~ 1 × 10^4 cells/wells were seeded into 96 well tissue culture plates and incubated for 48 hours. MCF-7 cells were treated with series of 10-100 µg/ml concentrations of Ag-NPs. The treated cells were incubated for 24 hours and 48 hours for cytotoxicity analysis. The cells were then subjected for MTT assay. The stock concentration (5mg/ml) of MTT-(3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, a yellow tetrazole was prepared and 100µl of MTT was added in each AgNps treated wells and incubated for 4 hours. Purple color formazone crystals were observed and these crystals were dissolved with 100µl of dimethyl sulphoxide (DMSO), and read at 620nm in a multi well ELISA plate reader (Thermo, Multiskan). OD value was subjected to sort out percentage of viability by using the following formula

$$\text{Percentage of viability} = \frac{\text{Mean OD value of experimental sample (AgNPs)}}{\text{Mean OD value of experimental control (un treated)}} \text{ X } 100$$

Determination of Ag-NPs induced apoptosis

Approximately 5µl of dye mixture (100mg/ml acridine orange (AO) and 100 mg/ml ethidium bromide (EtBr)) was mixed with 9 ml of cell suspension (1×105 cells/ml) on a clean microscopic cover slip. After incubation for 2-3 minutes cells were visualized under Epifluorescence microscope (Nikon Eclipse, Inc., Japan) at 40X magnification with excitation filter at 510-590nm.Percentage of apoptotic cells were determined by % of apoptotic cells

$$= \frac{\text{Total number of apoptotic cells}}{\text{Total number of normal \& apoptotic cells}} \text{ X } 100$$

STATISTICAL ANALYSIS

All the measurements were made in triplicate and all values were expressed as the mean ± standard error of the mean. Statistical significance was evaluated by one-way analysis of variance followed by Student's *t*-test (P<0.05), using the statistical package for social sciences (SPSS version 11; IBM Corporation, Armonk, NY, USA).

RESULTS AND DISCUSSION

Synthesis of Silver Nanoparticles

Human serum albumin is a most abundant plasma protein and it is widely used in Bio-nanotechnological applications. It possesses a zwitter ionic character at the isoelectric point (pH 7.4) wit exposed ionic groups (C and N Terminus) at the side chains of the globular protein, which are present in solution and are promising sites for binding cationic and anionicgroups[14].The general process for synthesis of Nanomaterials in the foam matrix involves the electrostatic complexation of metal ions with oppositely charged surfactant molecules, followed by foam generation and subsequent insitu chemical reaction[15]. HSA imparts greater flexibility by allowing the complexation of metal ions with opposite charges simultaneously. In this study we have prepared AgNps, by mixing HSA and Silver Nitrate solution and color change was observed by visual observation. The color of the $AgNO_3$/ HSA solution changed from colourless to light brown and eventually to dark brown (Figure 1).This colour change has indicated the formation of Ag-NPs in the solution. HSA solution without $AgNO_3$ did not show any colour changes.

Characterization of NPs

The formation of Ag Nanoparticles was further confirmed by using UV-visible spectroscopy, X-ray diffraction (XRD), Field Emission Scanning electron microcopy (SEM), energy-dispersive X-ray spectrometry (EDAX) and Fourier-transform infrared spectroscopy (FTIR). The synthesized Ag-NPs were characterized by UV-visible spectroscopy. UV-visible spectroscopy is an important and valuable technique for the characterization of NPs[16]. The UV-visible absorption spectra of the Ag-NPs were measured in the range of 300-800 nm. A strong and broad surface Plasmon peak located at 410 nm was observed and this showed the indication of Ag-NPs prepared using HSA (Figure 2). The strong surface Plasmon resonance centered at 400 nm has clearly indicated the formation of Ag-NPs, which were extremely stable, with no evidence of flocculation of the particles even after 3 months[17]. The band around 400 nm suggests that the particles were well dispersed without aggregation. Maximum intensity was achieved after 24 hours of the reaction, which indicates the complete reduction of the Ag^+ ions. An intense brown color of the reaction mixture further supports the complete reduction of Ag^+ ions and the formation of Ag-NPs.[18]Figure 3 shows XRD patterns for Ag-NPs synthesized using HSA. The XRD pattern indicated that the structure of Ag-NPs is face centered cubic[19]. In addition, all the Ag-NPs had a similar diffraction profile, and XRD peaks at 2θ of 38, 46.1, 64.9 and 77.4 could be attributed to the 111, 200, 220 and 311 crystallographic planes of the face-centered cubic silver crystals respectively[20]. The XRD pattern thus clearly illustrated that the Ag-NPs formed in this study were crystalline in nature. The main crystalline phase was silver, and there were no obvious other phases as

impurities were found in the XRD patterns. The average particle size of Ag-NPs calculated using the Debye-Scherrer equation

$$n = \frac{K\lambda}{\beta \cos\theta}$$

Where K is the Scherrer constant with value from 0.9 to 1 (shape factor), where λ is the X-ray wavelength (1.5418 Å), $\beta_{1/2}$ is the width of the XRD peak at half-height and θ is the Bragg angle. From the Scherrer equation, the average crystallite size of Ag-NPs for the sample at 24 hours of stirring are found to be 20-30 nm in size. Scanning Electron Microscopy was employed to visualize the size and shape of Ag-NPs. Figure 4 shows that the particles are relatively face centered cubical with the diameter of 20-30 nm. The Nanoparticles were not in direct contact even within aggregates, indicating stabilization of the Nanoparticles by HSA as capping agent. Figure 5 shows the clear elemental composition profile of the synthesized Ag-NPs. The intense signal at 3keV strongly suggests that Ag was the major element of these NPs, as it has optical absorption in this range due to the surface Plasmon resonance[21]. The other signals (carbon and oxygen) indicate the presence of tuber extract, which corresponds to the biomolecules that were capping over the Ag-NPs. The FTIR spectra were recorded (Figure 6) to identify the possible biomolecules responsible for the reduction of the Ag^+ ion and capping of the bioreduced Ag-NPs synthesized using HSA. After complete bioreduction of Ag^+, the solution was centrifuged at 15000 rpm for 20 minutes to isolate the Ag-NPs from proteins and other compounds present in the solution. The peaks near 3417 cm^{-1}, 2962 cm^{-1} and 2931 cm^{-1} assigned to amine stretching and aldehydic C-H stretching respectively. The weaker band 1653 cm^{-1} corresponds to amide I arising due to carbonyl stretch in proteins. The peak at 1037 cm^{-1} corresponds to C-N stretching vibration of the amine. The positions of these bands were close to that reported for native protein. This evidence suggests that the protein molecules could possibly perform the function of formation and stabilization of AgNps in aqueous medium.

MTT assay

Silver Nanoparticles as an antimicrobial agent, is gaining greater demand in medical applications. At the same time, there are only limited studies in the cytotoxic effects of AgNps synthesised using HSA, against cancer cell lines. MTT assay was used to assess the effect of AgNps on proliferation of breast cancer cells- MCF-7. There was no available data on the cytotoxicity of AgNps synthesized using HSA against breast cancer cell lines (MCF-7). In the present study, the dose dependent cytotoxicity was observed in AgNps treated MCF-7 cells (Figure 7) . Fifty percentage of cell death, which determines the inhibitory concentration (IC_{50}) value of synthesized AgNps against MCF-7 cells holds at 80 ng/mL in 24 hours and 40 ng/mL in 48 hours.

Determination of Ag-NPs induced apoptosis

The morphological changes were observed in AgNps treated breast cancer cells MCF-7 when compared with the untreated cells. The most recognizable morphological changes of AgNps treated cells observed in this study was the cytoplasmic condensation, cell shrinkage, production of numerous cell surface protuberances at the plasma membrane and aggregation of the nuclear chromatin into dense masses beneath the nuclear membrane . Fluorescence Microscopy Image of AgNps treated on MCF-7 cells exhibited characteristic apoptotic morphology, i.e., cell shrinkage, impaction of nuclei, and fragmentation of chromatin which is shown in Figure 8. The nuclei which had undergone condensation of chromatin fluoresced uniformly bright red. The control cells did not undergo these morphological changes and the nuclei as well as cytoplasm fluoresced uniformly green.

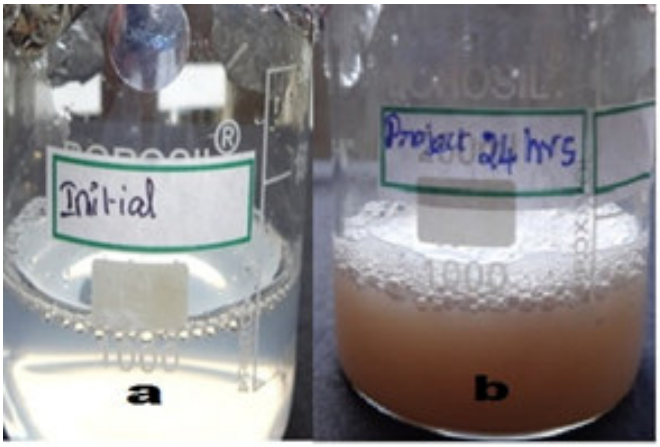

Figure1

Synthesis of silver Nanoparticles using HSA a) HAS foam matrix (Control) b) The color of the $AgNO_3$/ HSA solution changed from colourless to light brown (indication of Ag-NPs formation)

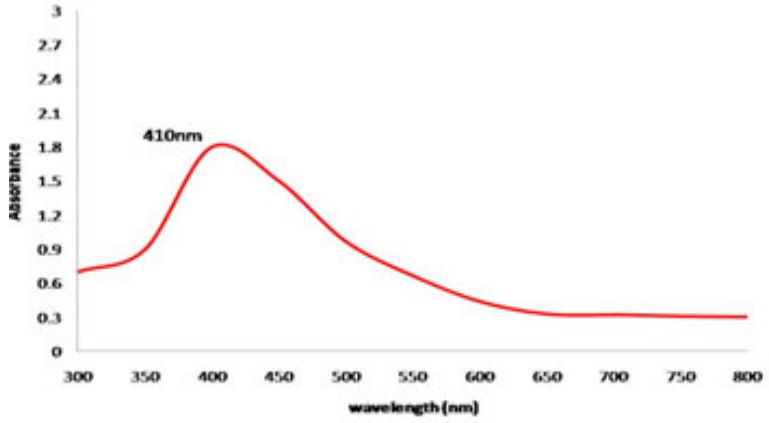

Figure 2

The ultraviolet-visible spectra of Ag-NPs. The absorption spectra of Ag-NPs exhibited a strong broad peak at 410nm, and observation of this band was attributed to surface Plasmon resonance of the particles.

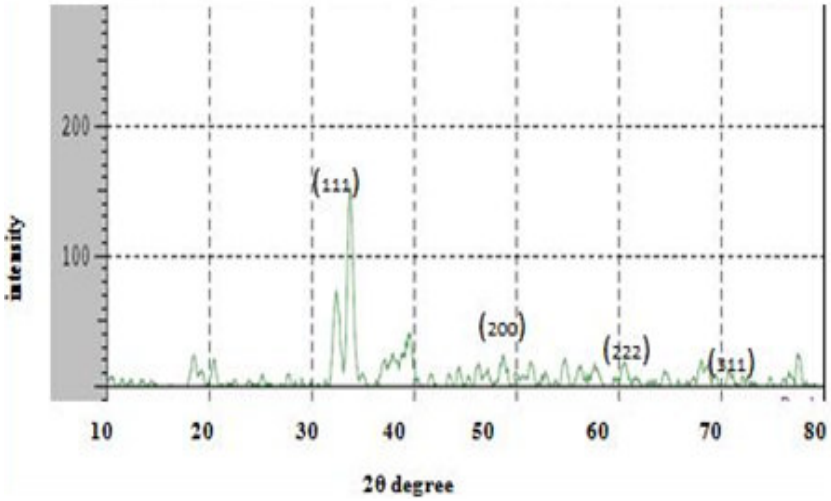

Figure 3

X-ray diffraction pattern of Ag-NPs synthesized using HSA. The diffractions at 38^0, 46.1^0, 64.9^0 and 77.4^0 can be indexed to 111, 200, 220 and 311 planes of the face-centered cubic Ag-NPs, respectively

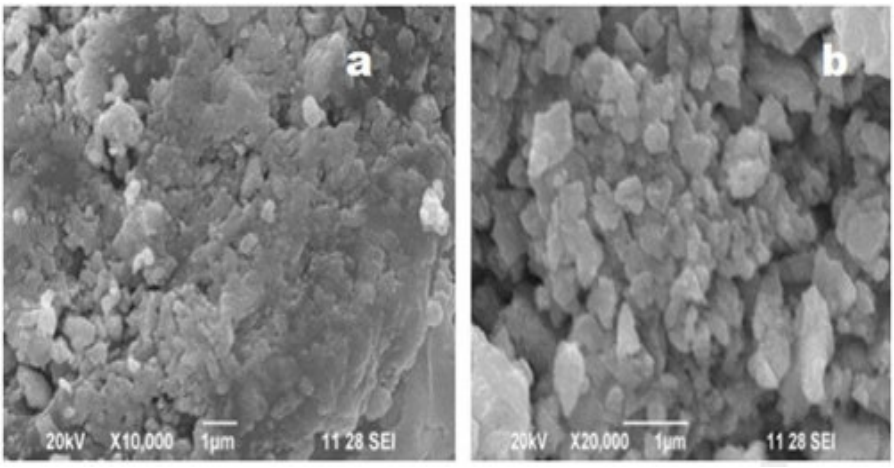

Figure 4

a &b shows Scanning Electron Microscopy image of Ag-NPs which are face centered cubical in shape

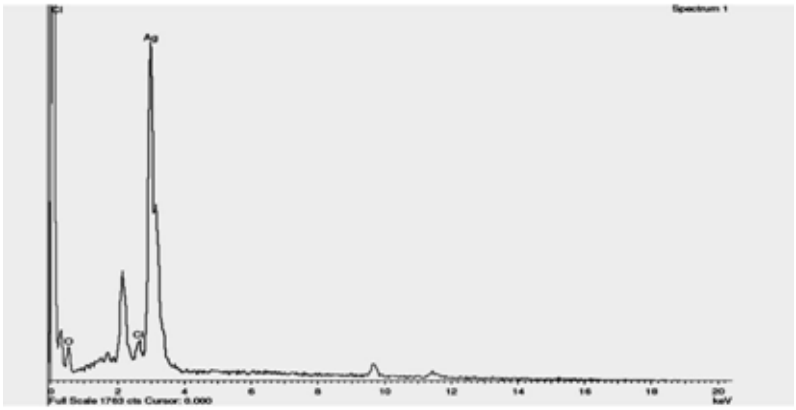

Figure 5
Energy dispersive X-ray fluorescence spectrometry spectra of synthesized Ag-NPs

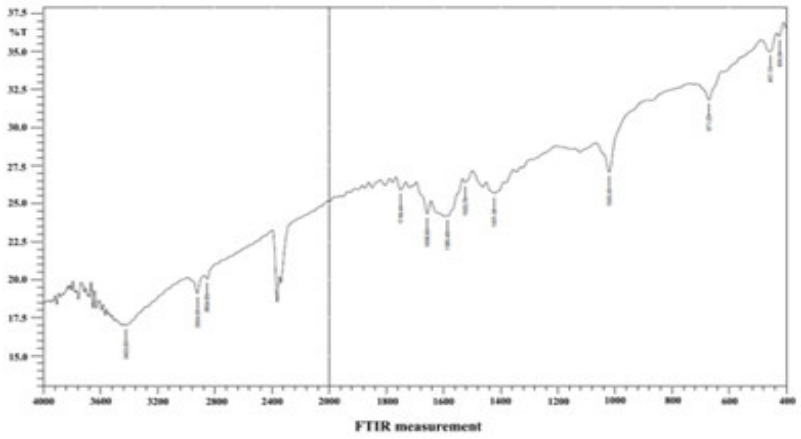

Figure 6
Fourier Transform Infra red spectra for Ag-NPs synthesized using HSA

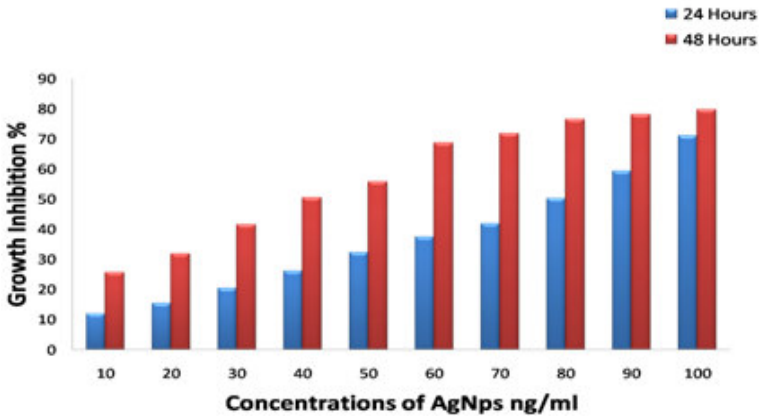

Figure 7
The in vitro cytotoxicity effect of AgNps against MCF-7 for 24 hrs & 48 hrs by MTT Assay. AgNps exhibited significant growth inhibition in a dose- and duration-dependent manner ($p<0.05$) & caused a marked decrease in cell growth.

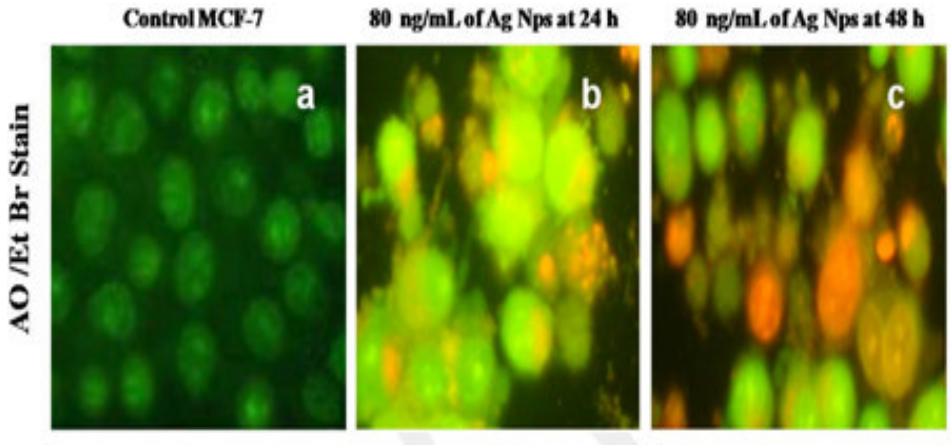

Figure 8a-8c
Fluorescence Microscopy Image of IC_{50} concentration of AgNPs treated on MCF-7. Cells exhibited characteristic apoptotic morphology, i.e., cell shrinkage, impaction of nuclei, and fragmentation of chromatin. The nuclei which had undergone condensation of chromatin fluoresced uniformly bright red. The control cells did not undergo these morphological changes and the nuclei as well as cytoplasm fluoresced uniformly green

CONCLUSION

The present study reports that the AgNps with an average size of 20-30 nm were synthesized by using HSA without using any harmful reducing or capping agents, as confirmed by UV-Visible spectrophotometer, XRD, SEM, EDAX and FTIR techniques. Further toxicity studies confirmed the potential cytotoxic effects of synthesized AgNps in MCF-7 cells. This study demonstrates the possibility of using AgNps to inhibit growth of cancer cells and in future their cytotoxicity potential offers new therapeutic treatment for cancer, arthritis and neovascularisation.

ACKNOWLEDGEMENTS

We gratefully acknowledge the University Grants Commission, Hyderabad, for sanctioning this minor research project and providing financial assistance. My heartfelt thanks to Sri Kaliswari College, Sivakasi for helping us to carry out as a part of our research work successfully. I am thankful to K.S.G College of Arts and Science, Coimbatore for encouraging me and providing laboratory facilities.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Bhimba BV, Kumari PR. Phytosynthesis of silver nanoparticles from the extracts of seaweed Ulva lactuca and its antimicrobial activity. Int J Pharm BioSci. 2014;5:666-77.
2. Simi CK, Abraham TE. Hydrophobic grafted and cross-linked starch nanoparticles for drug delivery. Bioprocess and biosystems engineering. 2007;30(3):173-80.
3. Cai W, Gao T, Hong H, Sun J. Applications of gold nanoparticles in cancer nanotechnology. Nanotechnology, science and applications. 2008; 2008(1).
4. Bader RA. The development of targeted drug delivery systems for rheumatoid arthritis treatment. INTECH Open Access Publisher; 2012.
5. Mody VV, Siwale R, Singh A, Mody HR. Introduction to metallic nanoparticles. Journal of Pharmacy and Bioallied Sciences. 2010 Oct 1;2(4):282.
6. Sekar RP. Formulation and evaluation of azathioprine loaded silver nanopartilces for the treatment of rheumatoid arthritis. Asian Journal of Biomedical and Pharmaceutical Sciences. 2013; 3(23):28.
7. Sevilla P, De Llanos R, Domingo C, Sánchez-Cortés S, Garcia-Ramos JV. SERS+ MEF of the anti-tumoral drug emodin adsorbed on silver nanoparticles. InBiOS 2010 Feb 11 (pp. 757714-757714). International Society for Optics and Photonics.
8. Mittal AK, Chisti Y, Banerjee UC. Synthesis of metallic nanoparticles using plant extracts. Biotechnology advances. 2013 Apr 30;31(2):346-56.
9. Kratz F. Albumin as a drug carrier: design of prodrugs, drug conjugates and nanoparticles. Journal of Controlled Release. 2008 Dec 18;132(3):171-83.
10. Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. Journal of controlled release. 2000 Mar 1;65(1):271-84.
11. Son YJ, Jang JS, Cho YW, Chung H, Park RW, Kwon IC, Kim IS, Park JY, Seo SB, Park CR, Jeong SY. Biodistribution and anti-tumor efficacy of doxorubicin loaded glycol-chitosan nanoaggregates by EPR effect. Journal of Controlled Release. 2003 Aug 28;91(1):135-45.

12. Maeda H. Tumor-selective delivery of macromolecular drugs via the EPR effect: background and future prospects. Bioconjugate chemistry. 2010 Apr 16;21(5):797-802.
13. Shameli K, Ahmad MB, Zamanian A, Sangpour P, Shabanzadeh P, Abdollahi Y, Zargar M. Green biosynthesis of silver nanoparticles using Curcuma longa tuber powder. International journal of nanomedicine. 2012;7:5603.
14. Huang XL, Catignani GI, Swaisgood HE. Micro-scale Method for Determining Foaming Properties of Protein. Journal of food science. 1997 Sep 1;62(5):1028-60.
15. Singh AV, Bandgar BM, Kasture M, Prasad BL, Sastry M. Synthesis of gold, silver and their alloy nanoparticles using bovine serum albumin as foaming and stabilizing agent. Journal of Materials Chemistry. 2005;15(48):5115-21.
16. Sastry M, Mayya KS, Bandyopadhyay K. pH Dependent changes in the optical properties of carboxylic acid derivatized silver colloidal particles. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 1997 Jul 2;127(1):221-8.
17. Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R, Sastry M. Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium oxysporum. Colloids and surfaces B: Biointerfaces. 2003 May 1;28(4):313-8.
18. Ahmad T, Wani IA, Manzoor N, Ahmed J, Asiri AM. Biosynthesis, structural characterization and antimicrobial activity of gold and silver nanoparticles. Colloids and Surfaces B: Biointerfaces. 2013 Jul 1;107:227-34.
19. Shameli K, Ahmad MB, Jazayeri SD, Shabanzadeh P, Sangpour P, Jahangirian H, Gharayebi Y. Investigation of antibacterial properties silver nanoparticles prepared via green method. Chemistry Central Journal. 2012 Jul 27;6(1):1.
20. Ahmad M, Shameli K, Darroudi M, Yunus W, Zin WM, Ibrahim NA. Synthesis and characterization of silver/clay/chitosan bionanocomposites by UV-irradiation method. American Journal of Applied Sciences. 2009;6(12):2030-5.
21. Banu AN, Balasubramanian C, Moorthi PV. Biosynthesis of silver nanoparticles using Bacillus thuringiensis against dengue vector, Aedes aegypti (Diptera: Culicidae). Parasitology research. 2014 Jan 1;113(1):311-6.

Reviewers of this article

G. Bakhya Shree M.S. (Research)

Coordinator and Trainer, Department of Biotechnology and Life Sciences, Dexter Academy, Madurai, Tamilnadu

Dr.A.R.Sumayya

HOD, Department of Biotechnology, KSG college of Arts & Science, Kamaraj Road, Upplipalayam P.O, Coimbatore - 15, Tamilnadu, India

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International Journal of Pharma and Bio sciences.

We sincerely thank the above reviewers for peer reviewing the manuscript