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Original Article

A Study on Antibacterial Activity of Silver Nanoparticles

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Abstract

This quasi experimental study was designed to evaluate antibacterial activity of chemically synthesized silver nanoparticles (AgNPs) from silver nitrate ($AgNO_3$) solutions on gram negative bacteria like *E.Coli* using disc diffusion method. Different concentrations of AgNPs, $AgNO_3$ and reference drug ciprofloxacin were used to find out the antibacterial activity which revealed that AgNPs possessed significant antibacterial effect compared to $AgNO_3$ solutions but relatively less antibacterial effect than that of ciprofloxacin. So chemical synthesis guided AgNPs may have some antibacterial effects.

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Introduction

Nanoparticle may be defined as particles of controlled size with at least one dimension less than 100nm (Behari 2010). That is nanoparticles are the particles between 1 to 100nm that behave as a whole unit in respect of its transport and properties. Nanoparticles are now being developed for a variety of biological applications such as medicines, antimicrobial agents, wound dressings, drug targeting and deliveries. Development of newer drug delivery system based on nanotechnology methods is being tried for conditions like cancer, diabetes, fungal infections, viral infections and gene therapy (Udapudi et al. 2012). The main advantage of this modality of treatment is specific targeting of the drug and enhanced safety profile. Nanotechnology has also found its use in diagnostic medicine as contrast agents like fluorescent dyes and magnetic nanoparticles (Surindran et al. 2009). Among metal nanoparticles silver have received considerable attention due to their attractive properties like size and shape depending optical, electrical and magnetic properties which can be incorporated into antimicrobial application, biosensor materials, cosmetic products and electronic components (Sileikaite et al. 2006). In most of the therapeutic applications it is the antimicrobial property that is being mostly explored. Numerous approaches have been used to prepare AgNPs. The most popular is the chemical method where glucose is used as reducing agent and starch as capping agent. Recently, the developments of antibiotic resistant or even multidrug resistant pathogens that possess a threat to clinical diseases have been a research of interest. Its inhibitory and bactericidal effects are due to huge increase in surface area, high fractions of surface atoms for the microbes to be exposed and increased catalytic activity that will lead to high antimicrobial activity as compared with bulk silver metals (Cho et al.2005; Kumar et al.2012). As a result, nanoparticles cause oxidative damage, inhibit cell transduction, cell lysis and ultimately death of the cell (Kim et al. 2011). Therefore, it is thought worthwhile to study the chemically synthesized AgNPs on Gram negative bacteria like

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E.coli in different strength using disc diffusion technique.

Material and Methods

Silver nitrate ($AgNO_3$), β-d glucose, starch were purchased from local market. The bacterial cultures of *E.coli* were obtained from the Department of Microbiology, Rajshahi Medical College . Antibiotic ciprofloxacin (Himedia, Mumbai, India) disc were purchased from local market. All glass wares and instruments (Conical flasks, measuring cylinders, beakers, petri plates and test tubes, micropipettes etc.) were purchased from local market. Deionized water was used throughout the experiment.

Preparation of Silver Nanoparticles: For synthesis of silver nanoparticles 200µl of 0.1M silver nitrate and 500µl of 0.1M glucose were placed into the conical flask using 1000 µl micropipette. Then 10ml of 0.2% starch was added into the flask. The mixtures of solutions were gently heated in a hot plate for 10 minutes. A change in color of the solution was obtained from colorless to yellow indicating the formation of silver nanoparticles.

Antibacterial Assay of Silver Nanoparticles: The antibacterial assays of different concentration of silver nanoparticles , AgNO3 solution and Ciprofloxacin were studied by standard disc diffusion method on gram-ve bacteria *E.coli*. Bacterial cultures of E.coli were obtained from Department of Microbiology, Rajshahi Medical College. Mueller-Hinton agar(MHA) was used to evaluate the sensitivity of nanoparticles. 0.1% inoculums (0.5 McFarland standard) suspension of overnight cultures of *E.coli* were swabbed uniformly and allowed to dry for 5 min. Sterile paper discs made of Whatman filter paper, 5 mm diameter dipped in 1.5mcg, 3.0mcg , 5.0mcg , 10mcg ,15mcg & 25mcg of silver nanoparticles & silver nitrate solution along with standard antibiotic ciprofloxacin containing discs were placed gently on agar plates with the help of a sterile forceps. The cultured agar plates were incubated at 37°C for 24 hrs. The zones of inhibition around the discs were measured after 24hrs of incubation with transparent ruler in millimeter.

Statistical Analyses: The results were calculated as means ± Standard deviation (SD). Data of zone of inhibition of *E.coli* by silver nanoparticles and $AgNO_3$ solution were compared using students *t*-test. A P-value less than 0.05 were considered statistically significant.

Observation and Results

When solution of glucose and starch was gently heated with aqueous solution of the $AgNO_3$, the color from colorless solution mixture started turning yellow just after 2-3 minutes of heating and after 10minutes it became yellowish brown . On the other hand, control solution (Deionized water, glucose and starch) did not develop color change after heating. . Absorption spectra of Ag nanoparticles formed in the reaction media had a sharp peak and absorbance maxima at 420 nm which indicates that size of synthesized AgNPs were below 30nm.

Table.1. Zone of inhibitions found in *E.coli* cultures.

Concentration of $AgNO_3$ & AgNP (mcg/disc)	Zone of inhibition (mm)			
	$AgNO_3$	AgNP	Ciprofloxacin	Control
1.5 mcg	6.1±0.32	7.5±1.17	20.2±1.56	00
3.0 mcg	6.1±0.42	7.9±1.27		
5.0 mcg	6.2±0.63	8.5±1.35		
10 mcg	7.2±1.31	10.1±1.38		
15 mcg	7.3±2.05	11.7±0.95		
25 mcg	7.0±2.18	12.3±2.18		

Cultures were treated with ciprofloxacin, AgNPs, $AgNO_3$ and control(gucose,starch and deionized water) solution. Results were expressed as Mean±SD.

The antibacterial effect of different concentrations of silver nanoparticles and $AgNO_3$ solution (1.5, 3.0, 5.0, 10, 15 & 25 mcg) were studied on gram-ve bacteria *E.coli* by disc diffusion method. Sensitivity of Ciprofloxacin as a standard antibiotic was also determined. Zone of inhibition caused by AgNPs , $AgNO_3$ and ciprofloxacin are shown in Table.1. Zone of inhibition created by reference antibiotic ciprofloxacin was 20.2mm, which was greater than created by AgNPs and $AgNO_3$. On the other hand, silver nanoparticles exhibited improved zone of inhibition than silver nitrate at all strength. Highest activity of AgNP was found at 25mcg. No zone of inhibition was found in control solution. . Statistical analysis proved that silver nanoparticles has got significant antibacterial activity ($p < .05$) as compared to $AgNO_3$ at all strength.

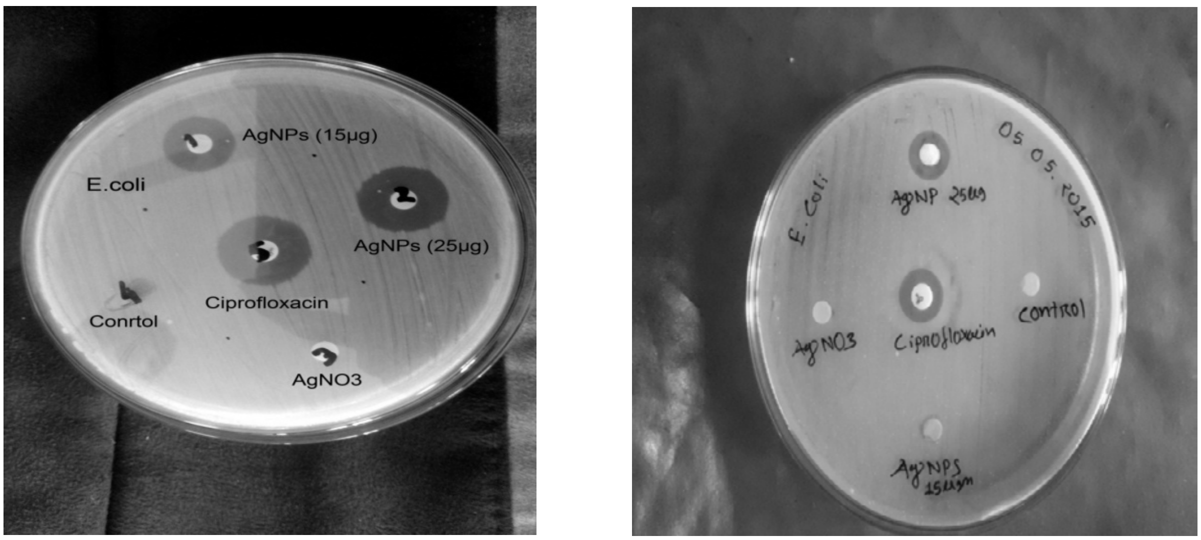

Fig. 2: Showing Antibacterial Effect of Different Concentrations of AgNPs, AgNO3, Ciprofloxacin & Control Solution. Clear zone of inhibitions created by silver nanoparticles , whereas the standard antibiotics Ciprofloxacin shows greater zone of inhibition as compared to the nanoparticles treated discs. Silver nitrate solution appeared less sensitive than silver nanoparticles and ciprofloxacin.

Discussion

It is well known that silver nanoparticles exhibit yellowish color in aqueous solution due to surface Plasmon vibration (Saini et al. 2013). The appearance of yellowish color in the solution suggests the formation of silver nanoparticles. Our observation is in agreement with the other studies reported earlier (Duran et al. 2007; Iravani and Zolfaghri 2013). In the present study, silver nanoparticles exhibited a single and well defined peak in the absorbance spectrum with maximum absorbance at 420nm which corresponds to characteristic surface Plasmon resonance of AgNPs. Pal, Song & Tak (2007) stated that only a single SPR band is expected in the absorption spectra of spherical nanoparticles, whereas anisotropic particles could give rise to two or more SPR bands depending on the shape of the particles. On the other hand, relatively narrow peak indicates that AgNPs were within a narrow size distribution. Therefore, the overall findings concluded that synthesis of silver nanoparticles using glucose as reducing agent and starch as capping agent were spherical and roughly spherical in shape and having narrow size.

In this study, silver nanoparicles showed concentration dependent activity. Though silver nanoparticles exhibited lower zone of inhibition than ciprofloxacin, AgNPs created significantly greater bacterial zone of inhibition and appeared highly sensitive compared to silver nitrate. The control solution of glucose, starch and distilled water did not show any antibacterial activity in *E.coli* cultures. The diameters of inhibition zone in the agar plate are summarized in Table 1. In a similar study, Abdullah & Hamid (2013) also evaluated concentration dependent activity of silver nanoparticles on Gram positive and Gram negative bacteria prepared from *Capsicum* sp. They obtained highest 10mm zone of inhibition in *E.coli* cultures using 7.5mcg/disc silver nano solution. In another similar study was done by Makwana & Shuklla (2014) showed that 8mm zone of inhibition in E.coli cultures was found at a lower dose of silver nanoparticles (1mcg/disc), which was possibly due to ultra small size and spherical shape of nanoparticles i.e. 5nm. The small size of metallic nanoparticles ensures that a significantly large surface area of the particles is in contact with the bacterial effluent. Because of ultra small size, AgNPs can easily reach the inner content of bacteria and they present a large and impressive surface area, enabling broad contact with bacteria. This could be the reason why they give the best antibacterial effect (Pal, Song & Tak 2007). The exact antibacterial effect of AgNPs is not clearly known, but Li et al. (2010) briefly described the mechanism of antibacterial effect of silver nanoparticles on *E.coli* as silver nanoparticles making a break through the permeability of outer membrane resulting in the leakage of cellular materials. SNPs also enter the inner membrane and inactivate respiratory dehydrogenase enzymes, thus inactivating respiratory chain and facilitating the generation of reactive oxygen species resulting inhibition of cellular growth and ultimately to cellular damage. Simultaneously, silver nanoparticles (SNPs) could affect some proteins and phospholipids and induce collapse of membrane, resulting cell decomposition and eventually cell death. Besides, Feng et al. (2000) also reported similar observations. They added that silver ions interact with thiol groups in protein which induce the inactivation of bacterial proteins and due to denaturation effect DNA molecules become condensed thus, losing their replication abilities. So in conclusion, studied guided synthesized silver nanoparticles using glucose as reducing agent and starch as capping agent showed significant antibacterial activity. Further studies should be done on wide range of bacterial strain involving Gram positive and Gram negative bacteria to evaluate the antibacterial effects of these synthesized NPs.

Conclusions

Nanotechnology stands for an important scientific advancement and can contribute with several benefits for human. In the present study, silver nanoparticles were successfully obtained from reduction of silver nitrate solution using glucose as reducing agent and starch as capping agent. UV-vis spectroscopy suggested the formation of uniform and spherical silver nanoparticles. From this study, silver nanoparticles was observed to have significant antimicrobial property. Silver nanoparticles produced by the method reported in this study using glucose as reducing agent and starch as capping agent have promising applications in biomedical, dental and pharmaceutical fields. Hence, effects and fates of this promising technology needs further studies to evaluate the potential risks of nanoparticles on environment.

References:

1. Abdullah AA & Hamid ZU. 2013, ‘Antimicrobial activity of silver nanoparticles from *Capsicum sp.* against *Staphylococcus sp, Bacillus sp., pseudomonas sp.* And *Escherichia coli*,’ *Journal of Biology, Agriculture and Healthcare,* vol. 3, no.7, pp.67-72.
2. Behari J. 2010,‘Principls of nanoscience: an overview’. *Indian Journal of Experimental Biology* , vol. 48, pp. 1008-1019.
3. Cho KH, Park JE, Osaka T & Park SG 2005, ‘The study of antimicrobial activity and preservative effects of nanosilver ingredient’, *Electrochil Acta* , vol.51, pp. 956-960.

4. Duran N, Marcato PD, De Souza GHI, Alves OL & Esposito E. 2007, 'Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment', *Journal of Biomedical Nanotechnology* , vol.3, pp. 203-208.
5. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN & Kim JO 2000. 'A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus', Journal of Biomedical Materials Research* vol.52, no.4 , pp.662–668.
6. Iravani S. & Zolfaghari B 2013. 'Green synthesis of silver nanoparticles using pinus elderica black extract', *Biomed Research International* , pp 1-5.
7. Kim SH, Lee HS, Ryu DS., Choi SJ & Lee DS 2011, 'Antibacterial activity of silver nanoparticles against S.aureus and E.coli,' *Korean Journal of Microbiology and Biotechnology* , vol. 39, no.1, pp. 77-85.
8. Kumar P, Selvi SS, Prabha AL, Selvaraj M, Rani LM, Suganthi P, Devi BS & Govindaraju M 2012 , 'Antibacterial activity and in-vitro cytotoxicity assay against Brine shrimp using silver nanoparticles sunthesized from *Sargassm Ilicifolium,' Digest Journal of Nanomaterials and Biostructures* , vol. 7, no. 4, pp.1447-1455.
9. Li WR, Xie XB, Shi QS, Zen HY, OU-Yang YS & Chen YB. 2010. 'Antibacterial activity and mechanism of silver nanoparticles on *Escherichia coli*,' *App Microbiol Biotechnol ,* vol. 85, pp. 1115-1122.
10. Pal S, Tak YK & Song JM 2007, 'Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium *Escherichia coli*,' *Applied and Environmental Microbiology*, vol. 73, no.6, pp.1712-1720.
11. Saini J, Kashyap D, Batra B, Kumar S, Kumar R & Malik Dk. 2013, 'Green synthesis of silver nanoparticles by using neem(Azadirachta Indica) and amla(Phyllanthus Emblica) leaf extract'*, Indian Journal of Applied Research* , vol.3, no.5, pp. 209-210.
12. Shukla A & Makwana BA 2014. 'Facile synthesis of silver nanoparticle and their potential application,*'American Journal of Nanoscience and Nanotechnology* , vol.2, no.4, pp.84-92.
13. Sileikate A, Prosycevas I, Puiso J, Juraitis A & Guobiene A 2006. 'Analysis of silver nanoparticles produced by chemical reduction of silver salt solution',*. International Journal of Material Science*, vol.12 , no. 4, pp. 287-291.
14. Surindiran A, Sandhiya S, Pradhan SC & Adithan C. 2009*,* 'Novel application of nanotechnology in medicin,' *Indian Journal of Medical research* , pp. 689-701.
15. Udapudi B, Naik P, Savadatti ST, Sharma R & Balgi S. 2012, 'Synthesis and characterization of silver nanoparticles,*' International Journal of Pharmacy and Biological Sciences ,* vol.2, no. 3, pp. 10-14.

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