



**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

**COMPARISON OF SILVER NANOPARTICLES WITH ROUTINELY USED
ANTIBIOTICS AND ITS EFFICACY AGAINST GRAM POSITIVE AND
GRAM NEGATIVE ORGANISM**

ANITHA M*, KARTHIKA JAYAKUMAR AND ARUN KUMAR J

Department of Microbiology, Shri Sathya Sai Medical College & Research Institute, Thiruporur,
Sri Balaji Vidyapeeth University, Tamil Nadu, India

*Corresponding Author: E Mail: animalar03@gmail.com

ABSTRACT

In this paper we report the antibacterial activity of silver nanoparticles synthesized from apple fruit extract. Our study is the first in vitro analysis with the involvement of Apple fruit extract. The novel silver nanoparticles exhibited a tremendous potential antibacterial activity against the multi drug resistant Gram positive and Gram negative bacterial strains. The zone of inhibition was extremely good showing a relatively large zone for both Gram positive and Gram negative bacterial strains.

The mechanism of prevention of bacterial growth by antibiotics is quite different from the mechanisms by which nanoparticles inhibit microbial growth. Therefore, nanoparticles have the potential to serve as an alternative to antibiotics and to control microbial infections. The obtained results suggest that Ag-NPs exhibit excellent bacteriostatic and bactericidal effect towards all clinical isolates. These results suggest that Ag-NPs could be used as an effective antibacterial agent.

Keywords: Multi Drug Resistant Strains, Silver Nanoparticles (Ag-Nps), Antibacterial Activity, Zone of Inhibition

INTRODUCTION

Due to the outbreak of the infectious diseases the development of antibiotic resistance the caused by different pathogenic bacteria and pharmaceutical companies and researchers are

searching for new antibacterial agents [1]. In the present scenario, nanoscale materials have emerged as novel antimicrobial agents owing to their high surface area to volume ratio and the unique chemical and physical properties [2-4].

Nanotechnology nowadays is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nanoscale level [5]. Silver nanoparticles (AgNPs) have emerged as an effective product from the field of nanotechnology. Over the last few years due to its good conductivity, chemical stability, catalytic and antibacterial activity [6-8], silver has gained much of the interest.

Silver and silver-based compounds have strong inhibitory and bactericidal effects and have been proved to be efficient compound in treating diseases from ancient times. Nanoparticles having larger surface area to volume ratio tend to pose higher antimicrobial activity. Based on these properties, Silver-nanoparticles (Ag-NPs) have been used in wide range of applications such as to prevent infection and silver ions work against bacteria in a number of ways: (a) Silver ions interact with the thiol groups of enzyme and proteins that are important for the bacterial respiration; (b) It alters DNA synthesis; (c) Silver ions are bound to the bacterial cell wall, altering the

function of the bacterial cell membrane, thus silver metal and its compounds are effective in preventing the infections of wound [9].

Human beings are often infected by microorganisms such as bacteria, molds, yeasts, and viruses present in their living environments. Because of the emergence and increase in the number of multiple antibiotic-resistant microorganisms and the continuing emphasis on health-care costs, many scientists have focused their research on the development of new methods for the synthesis of effective antimicrobial agents that will overcome the resistance of the microorganisms and which will also be cost-effective. Such problems and needs have led to resurgence in the use of silver-based antiseptics that may be linked to a broad-spectrum activity and considerably lower propensity to induce microbial resistance compared with those of antibiotics already available in market.

In particular, silver ions have long been known to exert strong inhibitory and bactericidal effects on Gram positive and Gram negative bacteria [10]. It can be expected that the high specific surface area and high fraction of surface atoms of nano-silver shapes will lead to high antimicrobial activity compared to bulk Ag metal. Surface area involves the increase of contact surface,

which is an important condition for the effects of silver nanoparticles [11]. Ag-NPs an effective antimicrobial agent might replace antibiotics in future medical treatment [12].

With the above background information, this present study was carried out for the efficacy and applicability of silver nanoparticles (Ag-NPs) in increasing the antibacterial activities against *S. aureus* and *E. coli*.

MATERIALS AND METHODS

Cultures

Twenty different bacterial strains were collected from the Diagnostic Microbiology lab, Shri Sathya Sai Medical College and Research Institute, Thiruporur, Tamil Nadu. Out of twenty, 12 strains were Gram positive bacteria, *Staphylococcus aureus* and 6 were Gram negative bacteria, *Escherichia coli*. The sub cultures were maintained on nutrient agar slants at 4°C. Overnight culture of the bacterial strain in the nutrient broth was used for the Ag-Nps studies [13].

Antibacterial Activity of Silver Nanoparticles

Silver nanoparticles synthesized from the extract of apple fruit were tested for their antibacterial efficacy against multi drug resistant strains of Gram positive bacteria, *S. aureus* and Gram negative bacteria *E. coli* following the method of Kirby-Bauer Disk Diffusion Susceptibility Test and the control

was maintained with routinely used antibiotics, Ciprofloxacin, Gentamicin, Ampicillin and Norfloxacin for comparing the antibacterial efficacy of silver nanoparticles on Gram positive and Gram negative bacteria [14, 15].

All the pure cultures of bacterial strains were sub-cultured in nutrient broth for 24hrs at 37°C. Twenty milliliter of the Mueller Hinton agar medium was poured into a petriplate on a horizontally leveled surface. Each bacterial strain was swabbed uniformly into the individual plates using sterile cotton swabs. Wells of 5mm diameter were made onto each bacterium inoculated agar plates using gel puncture. Using sterile micropipette, 20µl (0.005mg) of the sample of silver nanoparticle suspension was poured onto each of the wells at the centre of the plates. All the plates were incubated at 37°C. After 24 hours incubation, bactericidal activity was determined by measuring the zone of inhibition on the agar plate around the sample loaded wells.

RESULTS AND DISCUSSION

Out of the 20 bacterial strains taken for the study, 4 strains of *S. aureus* (Gram positive) and 3 strains of *E. coli* (Gram negative) were multi drug resistant (MDR) in nature (Table 1). These seven isolates were chosen for the study of antibacterial activity against silver nanoparticles. Antibiotics (Ampicillin,

Ciprofloxacin, Gentamicin) were used as control and the zone inhibition was observed (**Figure 1 and 2**).

When compared to the routine antibiotics such as Ampicillin, Ciprofloxacin, Gentamicin, the silver nanoparticles showed the maximum activity with the inhibition zone of 31 mm against Gram positive bacteria, *S. aureus* and 32 mm for Gram negative bacteria *E. coli* (**Figure 3 and Table 2**) where as the combination of Ag-NPs with the antibiotics (Ampicillin, Ciprofloxacin, Gentamicin) accentuated efficacy of antibiotic and silver nanoparticles with maximum zone of inhibition of 42 mm against Gram positive bacteria, *S. aureus* and 50 mm Gram negative bacteria *E. coli* (**Figure 4 and Table 2**).

The zone of inhibition using Hi media antibiotic zone scale clearly shows that all the bacterial strains which were resistant to antibiotics were highly susceptible to silver nanoparticles. This preliminary study clearly confirms that the silver nanoparticles are promising and effective antibacterial agent against the multidrug resistant bacteria (**Table 2**).

The addition of silver nanoparticles with antibiotics to these MDR strains not only exhibited sensitive zone but an extremely concentrated zone as shown in the (**Figure 4**). Thus our study proves combination of antibiotics with silver nanoparticles will enhance its antibacterial activity of the drugs.

Table 1: Bacterial Strains Used for the Study of Antibacterial Activity With Ag-NPs

Name of the strains	No of isolates	MDR strains
<i>Staphylococcus aureus</i>	12	4
<i>Escherichia coli</i>	8	3

Table 2: Zone of Inhibition Assay of Ag-NPs (With and Without Antibiotic) Against Different MDR Strains

Microorganisms	Antibiotic zone (mm)	Ag-Nps zone (mm)	Antibiotic+Ag-Nps zone (mm)
<i>S. aureus</i>	No inhibition	-	42
<i>S. aureus</i>	No inhibition	31	-
<i>E.coli</i>	No inhibition	32	-
<i>E.coli</i>	No inhibition	-	50



Figure 1: Kirby Bauer's Disk Diffusion Technique With Conventional Antibiotic Disc, Showing With Resistance to *Staphylococcus aureus*



Figure 2: Kirby Bauer's Disk Diffusion Technique With Conventional Antibiotic Disc, Showing With Resistance to *Escherichia coli*



Gram Positive Bacteria Gram Negative Bacteria Negative Control
Figure 3: Well Diffusion: Silver Nano Particles Without Antibiotic



Gram Positive Bacteria Gram Negative Bacteria Negative Control
Figure 4: Well Diffusion: Silver Nanoparticles With Antibiotic

CONCLUSION

Finally, we conclude that nanobiotechnology is an important area of research that deserves all our attention owing to its potential application to fight against multidrug-resistant microbes.

Our study reveals the antibacterial activity of Ag-NPs over multidrug resistant Gram positive and Gram negative bacteria. The zone of inhibition assay clearly shows that all the bacterial strains resistant to antibiotics were highly susceptible to silver nanoparticles. The combinations of Ag-Nps with antibiotics have extremely potent antibacterial activities against *S. aureus* and *E. coli* cells.

The significance of these results indicated that the Ag-NPs solutions have a good antibacterial activity for both *S aureus* and *E coli*. The surfaces cell walls of *S aureus* and *E coli* were disrupted by nanosilver shapes. It's an eye opener for the world which is thronged with Multi drug resistant strains facing the adversity of lack of sensitive antibiotics and our study may aid in the resolution of bringing in new drug which may become an effective bactericidal agent.

Thus, this preliminary study clearly confirmed that the silver nanoparticles synthesized from 'apple fruit extract' seems to be promising and effective antibacterial

agent against the multidrug resistant strains of bacteria

ACKNOWLEDGEMENT

The authors thank Dr. V. Narayanan, Professor, Department of Inorganic Chemistry, University of Madras, Guindy campus for helping in synthesizing silver nanoparticles at his laboratory.

REFERENCES

- [1] Rai M, Yadav A and Gade A, Silver nanoparticles as a new generation of antimicrobials, *Biotechnol. Advances*, 27, 2009, 76-83.
- [2] Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT and Yacaman MJ, The bactericidal effect of silver nanoparticles, *J. Nanotechnol.*, 16, 2005, 2346-2353.
- [3] Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, Kim YK, Lee YS, Jeong DH, Cho MH, Antimicrobial effects of silver, *Nanomedicine*, 3, 2007, 95-101.
- [4] Ansari MA, Khan HM, Khan AA, Malik A and Sultan A, Evaluation of antibacterial activity of silver nanoparticles, *Biol. and Med.*, 3 (2), 2011 (Special Issue), 141-146.

- [5] Albrecht MA, Evan CW and Raston CR, Green chemistry and the health implications of nanoparticles, *Green Chem.*, 8, 2006, 417-432.
- [6] Mukherjee P, Roy M, Mandal BP, Dey GK, Mukherjee PK, Ghatak J, Tyagi AK, Kale SP, Green synthesis of highly stabilized nanocrystalline silver particles by a non-pathogenic and agriculturally important fungus *T. asperellum*, *Nanotechnol.*, 19, 2008, 075-103.
- [7] Sondi I and Branka SS, Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria., *J. Colloid Interface Sci.* 275, 2004, 177-182.
- [8] Chen X and Schluesener HJ, Nanosilver: a nanoparticle in medical application, *Toxicol. Lett.*, 17, 2008, 1-12.
- [9] Geopriy G, Saravanan P and Gandhi NN, A novel approach for studying the combined Antimicrobial effects of AgNPs and Antibiotics, *Nanomaterials and Biostructures*, 6, 2011, 1557-1565.
- [10] Kim Soo-Hwan, Hyeong-Seon Lee, Deok-Seon Ryu, Soo-Jae Choi, Dong-Seok Lee, Antimicrobial activity of AgNPs against *Staphylococcus aureus* and *E.coli*. *Kor. J. Microbiol. and Biotechnol.*, 39, 2011, 77-85.
- [11] Sadeghi B, Jamali M, Sh. Kia, Synthesis and characterization of AgNPs for antimicrobial activity, *Int. J. Nano Dimension*, 1, 2010, 119-124.
- [12] Mitchell S and Laura S, Silver nanoparticles as a possible alternate to Antibiotic uses, Crandall University, 2003.
- [13] Koneman's, color atlas and textbook of Microbiology, 6th Edition, Lippincott Williams and Wilkins, 968-978.
- [14] Pankaj B and Sanjiv N, High prevalence of multi drug resistance from Kathmandu, Nepal, *BMC Res., Notes*, 5, 2012, 38.
- [15] Shirley A and Dayanand B Sreedhar, Antimicrobial activity of silver nanoparticles synthesized from novel *Streptomyces* species, *Digest J. Nanomat. and Biostructures*, 5, 2012, 447-451.