IJPSR (2012), Vol. 3, Issue 05 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 15 January, 2012; received in revised form 20 February, 2012; accepted 21 April, 2012

CHEMICALLY FABRICATED SILVER NANOPARTICLES ENHANCES THE ACTIVITY OF ANTIBIOTICS AGAINST SELECTED HUMAN BACTERIAL PATHOGENS

S. Thangapandiyan and P. Prema*

Post Graduate and Research Department of Zoology, V.H.N.S.N. College, Virudhunagar- 626 001, Tamil Nadu, India

Keywords:
Silver nanoparticles,
UV,
XRD,
SEM,
Antibacterial activity,
Synergistic effect

Correspondence to Author:

Dr. P. Prema

Assistant Professor, Research Department of Zoology, V.H.N.S.N. College, Virudhunagar- 626 001, Tamil Nadu, India

ABSTRACT

Due to the outbreak of infectious diseases caused by different pathogenic bacteria and the development of antibiotic resistance, the pharmaceutical companies and the researchers are now searching for new unconventional antibacterial agents. Nanotechnology represents a modern and innovative approach to develop new formulations based on metallic nanoparticles with antimicrobial properties. The potential bioactivity of chemically fabricated silver nanoparticles has been extensively studied. However, the antibacterial activity of silver nanoparticles individually or in combination with different antibiotics has not been demonstrated. In the present investigations, the effect of silver nanoparticles on the antibacterial activity of different antibiotics was evaluated against selected human bacterial pathogens such as *Staphylococcus aureus, Streptococcus epidermis, Escherichia coli, Pseudomonas aeruginosa,* and *Bacillus cereus* by disc diffusion method. In the presence of sub - inhibitory concentration of silver nanoparticles (100µL/disc), the antibacterial activities of all antibiotics are increased from 1 mm to 10 mm. The maximum fold increase was noticed for *vancomycin* against *Pseudomonas aeruginosa* (66.67%), *Escherichia coli* (62.50%), and *Staphylococcus aureus* (46%) followed by *rifampicin* against *Bacillus cereus* (66.67%) and kanamycin against *Streptococcus epidermis* (25%). These results signify that the silver nanoparticles showed potential antibacterial action of ß-lactams, glycopeptides, aminoglycosides, sulphonamides suggesting a possible utilization of silver nanocompounds in combination therapy against selected pathogens used in the experiment.

INTRODUCTION: The emergence of bacterial resistance to antibiotics and its dissemination pose serious threads to human beings. One of the field in which nanotechnology find extensive applicants in nanomedicine, an emerging new field which is an outcome of fusion of nanotechnology and medicine. Nanotechnology is expected to open new avenues to fight and prevent diseases using atomic scale tailoring of materials.

Rapid development of Bio-nanotechnology and material research leads to a new way in combating bacteria and searching specific properties of nanomaterials. Nanotechnology can improve our understanding of living cells and molecular level interactions. A number of nanoparticles based therapeutics has been approved clinically for infections, vaccines and diseases [1].

Nanostructured materials are attracting a great deal of attention because of their potential of achieving specific processes and selectivity, especially in biological and pharmaceutical applications [2-4]. Gold, silver and copper have been used mostly for the synthesis of stable dispersion of nanoparticles [5, 6]. A unique characteristic of these synthesized metal nanoparticles is that a change in the absorbance or wavelength gives a measure of the particle size, shape and interparticle properties [7].

Nanomaterials are called "a wonder of modern medicine". It is stated that antibiotics kill perhaps a half dozen different disease causing organisms but nanomaterials can kill some 650 cells [8]. Among the various metal nanoparticles, silver nanoparticles have received substantial attention for various reason that silver is an effective antimicrobial agent and exhibits low toxicity [9, 10]. The application of silver nanoparticles in medical industry as topical ointments to prevent infection against burn and open wounds [11]. Silver salts and silver compounds have been used in different biomedical fields, especially in burn treatment [12].

Silver nanoparticles are widely used for its unique properties in catalysis, chemical sensing, biosensing, photonics, electronic and pharmaceuticals [13] and biomedicine especially for antibacterial agent [14] and antiviral agents [15]. Silver nanoparticles have a great potential for use in biological including antimicrobial activity [16].

Antimicrobial capability of silver nanoparticles allows them to be suitably employed in numerous household products such as textiles, food storage containers, home appliances and medical devices [17]. Silver is an effective antimicrobial agent exhibits low toxicity [18]. The antibacterial activity of silver species has been well known since ancient times [19]. The most important application of silver and silver nanoparticles is in medical industry such as tropical ointments to prevent infections against burn and open wounds [11].

Our aim in the present contribution was to synthesis and characterization of Ag-NPs by chemical reduction method and to investigate the synergistic effect of Ag-NPs combined with various antibiotics against selected human bacterial pathogens.

MATERIALS AND METHODS:

Materials: Silver nitrate ($AgNO_3$), Trisodium citrate ($C_6H_5O_7Na_3$) and commercial antibiotic discs purchased from Himedia (P) Ltd, Mumbai, were used as starting materials without further purification. Milli-Q water was used throughout the experiment.

Methods:

Preparation of Silver Nanoparticles: The silver nanoparticles were prepared using chemical reduction method [20]. In this method, 150 ml of $1X10^{-3}$ M $AgNO_3$ solution was heated to boiling. To this solution 5 ml of 1% trisodium citrate was added drop by drop. During this process solution was mixed vigorously. Reaction mixture was heated until color change is evident (pale yellow). Then, it was removed from heating element and stirred until cooled to room temperature.

Mechanism of reaction could be expressed as follows:

$$4Ag^+ + C_6H_5O_7Na_3 + 2H_2O \rightarrow 4Ag^0 + C_6H_5O_7H_3 + 3Na^+ + H^+ + O_2 \uparrow$$

The solution was washed three times in double distilled water and once in ethanol solution. The supernatant solution was discarded and particles were dried in hot air oven at 60^{o}C. The dried particles were taken for further analysis.

Characterization of Silver Nanoparticles:

Visual inspection: The reduction of metal ions was roughly monitored by visual inspection of the solution by color change.

UV-Vis Spectroscopy: UV-Vis spectroscopy of silver nanoparticles was performed on Shimadzu dual beam spectrophotometer (model UV-2000 Shimadzu) operated at a resolution of 1nm.

X- ray Diffraction: The crystallographic analysis of the samples was performed by powder X-ray diffraction. The X-ray diffraction patterns were recorded in the scanning mode on an X'pert PROPAN analytical instrument operated at 40 kV and a current of 30 mA with Cu α radiation (λ=1.54060 A$^\circ$). The diffraction intensities were recorded from 35° to 79.93° in 2θ angles. The diffraction intensities were compared with the standard JCPDS files.

The software gave the information about the crystal structure of the particle and the average size of the particles can be estimated using the Debye-Scherer equation

$$D = k\lambda / \beta \cos\theta,$$

Where D is the thickness of the nanocrystal, 'k' constant, 'λ' wavelength of X-rays, 'β' width of the half-height of the reflection after correction for the instrumental broadening at Bragg's angle 2θ, 'θ' Bragg's angle

Scanning Electron Microscopy: Morphology of the synthesized silver nanoparticles was investigated with the Scanning Electron Microscope (JSM 35 CF JEOL) operated at a resolution of 60 A° at 15kv magnification of 5.0k. The scale was about 32mm to 3.6µm.

Antibacterial Activity of Silver Nanoparticles :

Bacterial Cultures: In the present experiment, the following human bacterial pathogens namely *Staphylococcus aureus, Streptococcus epidermis, Escherichia coli, Pseudomonas aeruginosa,* and *Bacillus cereus* were grown on nutrient agar plate and maintained in the nutrient agar slants at 4°C. Overnight culture in the nutrient broth was used in the experimental.

Antibacterial Activity of Silver Nanoparticles: The antibacterial activity of synthesized silver nanoparticles was evaluated using the agar-well diffusion method. Pure cultures were subcultured in nutrient broth for 24hrs at 37°C. A 20ml volume 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 6mm diameter were made onto each bacterium inoculated agar plates using gel puncture. 100µl of silver nanoparticle suspension was introduced into the corresponding wells using sterile micropipette. The bactericidal activity was determined by a clear inhibition zone around the sample loaded well after incubation the plates at 37°C for 24 hours.

Synergistic effect of Silver Nanoparticles with Commercial Antibiotic Discs: Synergistic effect of silver nanoparticles was tested using disc diffusion method. The following commercial antibiotics such as *Amikacin (Ak), Ampicillin (A), Chloramphenical (C), Gentamycin (G), Kanamycin (K), Penicillin (P), Rifampicin (R), Streptomycin (S), Tetracycline (T), Tobramycin (TB),* and *Vancomycin (V)* were used. Pure cultures were subcultured in nutrient broth for 24hrs at 37°C. Nutrient broth was used as source material for culturing the test pathogens.

Each strain was swabbed uniformly into the individual plates of Mueller hinton agar using sterile cotton swabs. Antibiotic discs with and without silver nanoparticles were placed. Results were recorded by measuring the diameter of inhibitory zone in mm after 24hrs of incubation at 37°C.

Results and Discussion

Visual inspection of Silver Nanoparticles: The appearance of pale yellow color colloidal solution indicates the formation of silver nanoparticles in the reaction mixture is shown in **Fig. 1**. The color of the solution was due to the excitation of surface Plasmon vibration in the silver nanoparticles.

FIG. 1: FRESHLY PREPARED YELLOW COLLOIDAL SILVER BY CHEMICAL REDUCTION METHOD

UV-Vis Spectroscopy: UV-Vis absorption spectra have been proved to be quite sensitive to the formation of silver colloids because silver nanoparticles exhibit an intense absorption peak due to the surface Plasmon excitation around 428 nm is depicted in **Fig. 2** which clearly indicates the formation of silver nanoparticles in solution.

Similarly earlier reports revealed that the intensity of the absorbance was at between 350-450nm [21, 22].

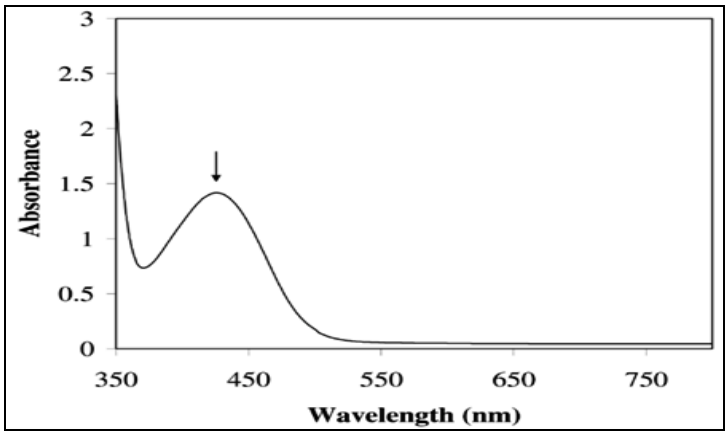

FIG. 2: UV-VIS ABSORPTION SPECTRA OF SILVER NANOPARTICLES SYNTHESIZED BY CHEMICAL REDUCTION METHOD

X-ray Diffraction: The intensive diffraction peak at a 2θ value of 38.18° from the (111) lattice plane of face centered cubic (fcc) unequivocally indicates that the particles are made of pure silver. Three additional bands are observed at 44.32°, 64.50° and 77.05° which corresponds to the (200), (220), and (311) planes of silver respectively is predicted in **Fig. 3**. The Bragg's peak position and their intensities were compared with standard JCPDS files. The result shows that the particles have a cubic structure. The size of the silver nanoparticles was found to be 25 nm. The observed diffraction pattern coincides with the earlier report [21].

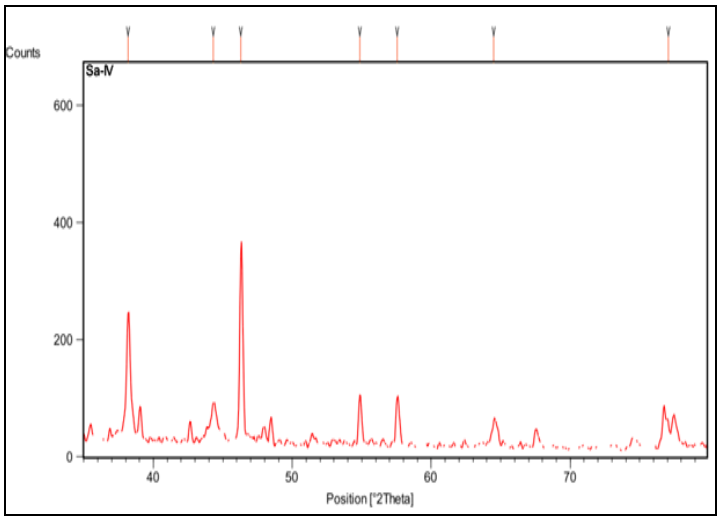

FIG. 3: X-RAY DIFFRACTION OF SILVER NANOPARTICLES SYNTHESIZED BY CHEMICAL REDUCTION METHOD

Scanning Electron Microscopy: The scanning electron micrograph of silver nanoparticles is depicted in **Fig. 4**. The scanning electron micrograph shows that the particles were spherical in nature. The spherical natured SEM image of the synthesized particles showed 90% similarity with the earlier studies [22].

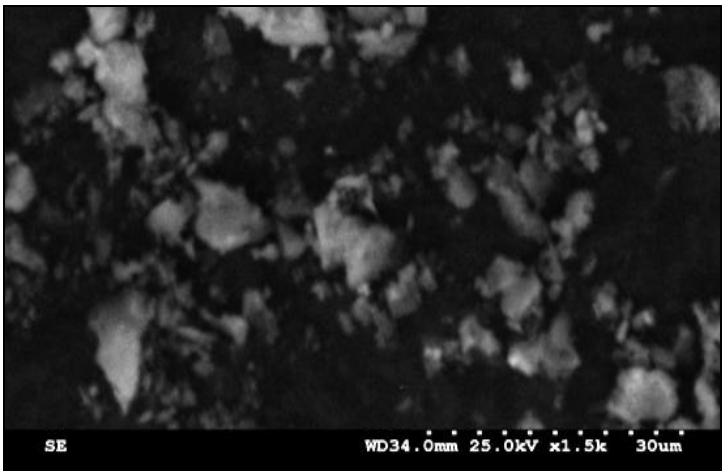

FIG. 4: SCANNING ELECTRON MICROGRAPH OF SILVER NANOPARTICLES SYNTHESIZED BY CHEMICAL REDUCTION METHOD

Antibacterial acitivity of Silver Nanoparticles: Silver nanoparticles produced highest inhibition zone against *E. coli* followed by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Streptococcus epidermis* is given in **Fig. 5**. The recorded zone of inhibition against pathogens was ranged from 10mm to 20mm is represented in **Table 1**. The cellular membranes in bacterial cells which contain pores in nanometer range. The nanoparticles which have a size less than the pores in the bacteria have a unique property of crossing the cell membrane without any hindrance.

Earlier reports proved that the incorporation of AgNPs into the bacterial cell membrane and make significant changes and damage by formation of pits on their surfaces [10]. A bacterial membrane with this morphology exhibits a significant increase in permeability, leaving the bacterial cells incapable of properly regulating transport through the plasma membrane and, finally, causing cell death. Silver nanoparticles exhibited antibacterial properties against bacterial pathogens with close attachment of the nanoparticles themselves with the microbial cells.

The mechanism of inhibitory action of silver ions on microorganisms is partially known. It is believed that DNA loses its replication ability and cellular proteins become inactivated on Ag+ treatment [23]. The Ag+ binds to functional groups of proteins, resulting in protein denaturation [24]. Inhibition of respiratory process [25] DNA unwinding [26] Inhibition of cell division and damage of cell envelops [27].

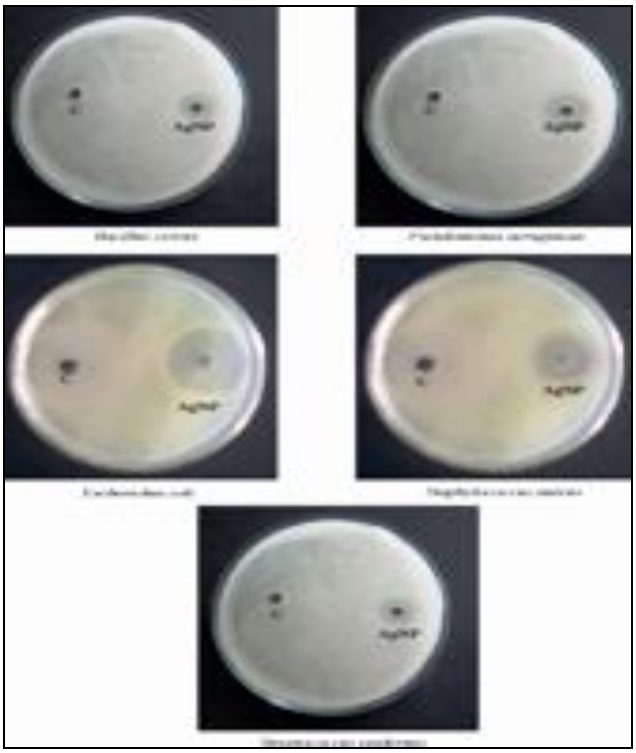

FIG. 5: ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES AGAINST BACTERIAL PATHOGENS

TABLE 1: ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES AGAINST SELECTED HUMAN BACTERIAL PATHOGENS

Bacterial pathogens	Zone of inhibition (mm) (Mean ± S.D)
Staphylococcus aureus	18 ± 0.12
Streptococcus epidermis	10 ± 0.10
Escherichia coli	20 ± 0.10
Pseudomonas aeruginosa	13 ± 0.23
Bacillus cereus	10 ± 0.11

Synergistic Effect of Silver Nanoparticles with Commercial Antibiotics: The combined effect of different antibiotics with and without silver nanoparticles was investigated against gram-positive and gram-negative bacterial strains used in this experiment is shown in **Fig. 6**.

The zone of inhibition (mm) for all antibiotics has increased in the presence of silver nanoparticles which are given in **Table 2-6.** The resultant zone was compared with standard chart recommended by NCCLS [28]. The maximum fold increase was found while using *vancomycin* as an antibiotic against *P. aeruginosa* (66.67%), *E.coli* (62.50%), *S.aureus* (46%) followed by *rifampicin* against *B.cereus* (66.67%) and *kanamycin* against *S.epidermis* (25%).

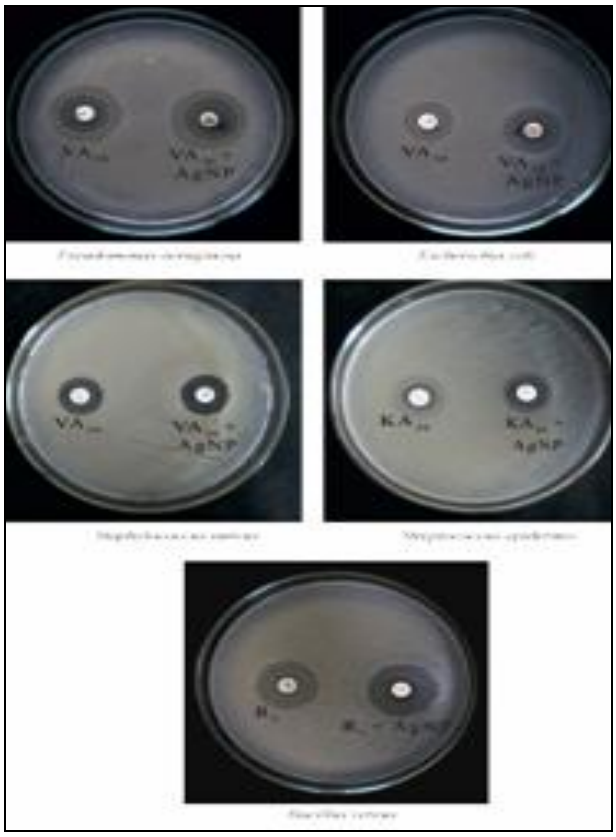

FIG. 6: SYNERGISTIC EFFECT OF SILVER NANOPARTICLES WITH COMMERCIAL ANTIBIOTICS

TABLE 2: AVERAGE (MEAN±S.D.) ZONE OF INHIBITION (MM) OF DIFFERENT ANTIBIOTICS WITH AND WITHOUT SILVER NANOPARTICLES AGAINST *STAPHYLOCOCCUS AUREUS* AS TEST STRAIN

Antibiotics (µg/disc)	Zone of Inhibition (mm)		
	Silver nanoparticles without Antibiotics	Silver nanoparticles with Antibiotics	Fold Increase (b –a/a) 100 %
Amikacin	24.00 ± 0.63	30.00 ± 2.82	25.00
Penicillin	7.00 ± 1.41	9.00 ± 1.41	28.00
Ampicillin	30.00 ± 2.82	33.00 ± 2.28	10.00
Streptomycin	17.00 ± 1.41	20.00 ± 2.19	17.65
Tobramycin	18.00 ± 2.82	20.00 ± 2.19	11.11
Vancomycin	13.00 ± 2.28	19.00 ± 1.26	46.00
Rifampicin	10.00 ± 1.78	13.00 ± 1.41	30.00
Kanamycin	14.00 ± 1.26	16.00 ± 2.61	14.29
Chloramphenicol	15.00 ± 1.41	20.00 ± 2.19	33.33
Gentamycin	18.00 ± 2.82	25.00 ± 5.11	38.00
Tetracycline	17.00 ± 1.41	22.00 ± 2.28	29.41

TABLE 3: AVERAGE (MEAN ± S.D.) ZONE OF INHIBITION (MM) OF DIFFERENT ANTIBIOTICS WITH AND WITHOUT SILVER NANOPARTICLES AGAINST *Streptococcus epidermis* AS TEST STRAIN

Antibiotics (µg/disc)	Zone of Inhibition (mm)		Fold Increase (b –a/a) 100 %
	Antibiotics without Silver nanoparticles	Antibiotics with Silver nanoparticles	
Amikacin	17.00 ± 1.41	20.00 ± 2.82	17.65
Penicillin	9.00 ± 2.60	12.00 ± 2.28	33.33
Ampicillin	10.00 ± 1.78	14.00 ± 1.26	40.00
Streptomycin	20.00 ± 2.28	25.00 ± 4.47	25.00
Tobramycin	15.00 ± 1.41	18.00 ± 2.82	20.00
Vancomycin	15.00 ± 1.41	21.00 ± 2.31	40.00
Rifampicin	12.00 ± 1.41	15.00 ± 2.00	25.00
Kanamycin	11.00 ± 1.41	17.00 ± 2.00	54.54
Chloramphenicol	25.00 ± 4.47	26.00 ± 1.85	4.00
Gentamycin	25.00 ± 3.22	26.00 ± 1.86	4.00
Tetracycline	24.00 ± 2.83	25.00 ± 3.68	4.17

TABLE 4: AVERAGE (MEAN±S.D.) ZONE OF INHIBITION (MM) OF DIFFERENT ANTIBIOTICS WITH AND WITHOUT SILVER NANOPARTICLES AGAINST *PSEUDOMONAS AERUGINOSA* AS TEST STRAIN

Antibiotics (µg/disc)	Zone of Inhibition (mm)		Fold Increase (b –a/a) 100 %
	Antibiotics without Silver nanoparticles	Antibiotics with Silver nanoparticles	
Amikacin	20.00 ± 2.19	21.00 ± 1.02	5.00
Penicillin	7.00 ± 1.41	11.00 ± 3.16	57.14
Ampicillin	10.00 ± 2.28	14.00 ± 1.26	40.00
Streptomycin	20.00 ± 2.19	25.00 ± 3.16	12.00
Tobramycin	14.00 ± 1.26	21.00 ± 1.02	50.00
Vancomycin	12.00 ± 1.41	20.00 ± 2.19	66.67
Rifampicin	15.00 ± 2.00	17.00 ± 1.41	13.33
Kanamycin	17.00 ± 2.82	22.00 ± 2.28	29.41
Chloramphenicol	20.00 ± 2.19	25.00 ± 3.68	25.00
Gentamycin	17.00 ± 1.41	20.00 ± 2.19	17.65
Tetracycline	20.00 ± 2.19	25.00 ± 3.60	25.00

TABLE 5: AVERAGE (MEAN±S.D.) ZONE OF INHIBITION (MM) OF DIFFERENT ANTIBIOTICS WITH AND WITHOUT SILVER NANOPARTICLES AGAINST *ESCHERICHIA COLI* AS TEST STRAIN

Antibiotics (µg/disc)	Zone of Inhibition (mm)		Fold Increase (b –a/a) 100 %
	Antibiotics without Silver nanoparticles	Antibiotics with Silver nanoparticles	
Amikacin	25.00 ± 0.63	28.00 ± 1.41	12.00
Penicillin	10.00 ± 2.28	14.00 ± 1.41	40.00
Ampicillin	12.00 ± 2.00	17.00 ± 1.41	41.67
Streptomycin	25.00 ± 3.54	28.00 ± 3.16	12.00
Tobramycin	15.00 ± 2.00	20.00 ± 2.28	33.33
Vancomycin	8.00 ± 2.00	13.00 ± 1.26	62.50
Rifampicin	10.00 ± 2.28	15.00 ± 2.00	50.00
Kanamycin	13.00 ± 3.22	20.00 ± 2.19	46.66
Chloramphenicol	13.00 ± 2.82	20.00 ± 2.31	53.84
Gentamycin	20.00 ± 2.19	25.00 ± 3.68	25.00
Tetracycline	15.00 ± 1.41	25.00 ± 2.19	33.33

TABLE 6: AVERAGE (MEAN±S.D) ZONE OF INHIBITION (MM) OF DIFFERENT ANTIBIOTICS WITH AND WITHOUT SILVER NANOPARTICLES AGAINST *BACILLUS CEREUS* AS TEST STRAIN

Antibiotics (µg/disc)	Zone of Inhibition (mm)		Fold Increase (b –a/a) 100 %
	Antibiotics without Silver nanoparticles	Antibiotics with Silver nanoparticles	
Amikacin	20.00 ± 2.28	22.00 ± 1.96	10.00
Penicillin	8.00 ± 0.63	10.00 ± 2.28	25.00
Ampicillin	12.00 ± 1.41	15.00 ± 2.00	25.00
Streptomycin	16.00 ± 2.61	23.00 ± 2.00	43.75
Tobramycin	12.00 ± 1.41	17.00 ± 1.41	41.67
Vancomycin	19.00 ± 4.00	23.00 ± 2.00	21.05
Rifampicin	12.00 ± 1.41	20.00 ± 2.28	66.67
Kanamycin	22.00 ± 2.28	25.00 ± 3.68	13.64
Chloramphenicol	18.00 ± 4.00	28.00 ± 2.82	55.56
Gentamycin	21.00 ± 2.00	27.00 ± 1.41	28.57
Tetracycline	12.00 ± 2.00	19.00 ± 2.31	58.33

The synergistic effect may be due to certain complex formation which becomes more effective in the inhibition of a particular species of microorganisms either by inhibiting the cell wall synthesis or by causing its lysis or death. In this present evidence to control particular disease, *invitro* experiments should be carried out various antibiotics combined with silver nanoparticles. The distinct difference was observed between the inhibitory zones produced by dics with and without silver nanoparticles. The diameter of the zone was increased when the nanoparticles were present. This condition may be due to its accumulation in the bacterial membrane.

A membrane with such morphology exhibits a significant increase in permeability, resulting in death of the cell [10]. The increase in synergistic effect may cause the bonding reaction between antibiotic and nanosilver. The antibiotic molecules contain many active groups such as hydroxyl and amido groups, which reacts easily with nanosilver by chelation. The AgNPs-ampicillin complex reacts with DNA and prevents DNA unwinding, which results in more serious damage to bacterial cells [29].

These nontoxic silver nanomaterials can be prepared in a simple and cost-effective manner, may be suitable for the formulation of new types of bactericidal materials and may be solve the problem of the emergence of spread of *in vitro* antibiotic resistance. Investigations have been carried out on the biological activity of AgNPs; however, the effect of nanoparticles on the activities of antibiotics has not been concluded. The synergistic antibacterial effect with the combination of nanosilver and antibiotics has more potential. Here we present a possible explanation for the enhancement of the synergistic antibacterial mechanism.

This research provides helpful insight into the development of new antimicrobial agents. However, future studies on the biocidal influence of nanomaterial on other Gram-positive and Gram-negative bacteria are necessary to fully evaluate its possible use as a new bactericidal material.

CONCLUSION: Nanobiotechnology is an important area of research that deserves all our attention owing to its potential application to fight against microbes.

In conclusion, the synthesized silver nanoparticles by chemical reduction method are spherical shaped by SEM and the average size about 25nm by X-ray diffraction. We put up an innovation point which is to utilize the synergistic antibacterial effects by screening. We speculate and reported the antibacterial activity of AgNPs over multidrug resistant Gram-positive and Gram-negative bacteria.

This research though preliminary, provides helpful insight to the development of novel antimicrobial nanoparticles. To elucidate the mechanism of the synergistic effect of chemically synthesized AgNP, more elaborate experimental evidences will be needed.

ACKNOWLEDGEMENT: The authors are grateful for the financial support provided by University Grant Commission (Major Project F.No. 39/630/2010 (SR)) and thank V.H.N.S.N. College Managing Board, Virudhunagar, for providing facilities and Allagappa University, CECRI, Karaikudi for technical assistance.

REFERENCE:

1. R. Malhorta, Curr. Sci.,2,140(2010).
2. X. Wu, H. Liu, J. Liu, K.N. Haley, J.A.Treadway and J.P. Larson, Nat. Biotechnol 1, 41 (2003).
3. J.D. Fortner, D.Y. Lyon, C.M. Sayes, A.M. Boyd, J.C. Falkaer, E.M. Hotze and Alanany, Environ. Sci. Technol., 11, 4307 (2005).
4. P. Li, J. Li, Q. Wu and J. Li, J. Nanotechnol., 9, 1912, 2005.
5. A.M. Smith, H. Duan, M.N. Rhynaer, G. Ruan and S.A. Nie, Phys. Chem. Chem. Phys., 3895 (2006).
6. G.J. Kearns, E.W. Foster and J.E. Hutchison, Anal. Chem., 1, 298 (2006).
7. P. Mulvaney, Langmuir,12, 780 (1996).
8. T. Sungkaworn, W. Triampo, P. Nalakarn, D. Triampo, I.M. Tang and Y. Lenbuey, Int. J. Biomed. Sci., 1, 67 (2007).
9. D. Jain, H.K. Daima, S. Kachhuaha and S.L. Kothjari, Digest J. Nanomater. Biostruct., 3, 557 (2009).
10. I. Sondi and B. Salopek-Sonndi, J. Colloid. Interf. Sci., 1, 177 (2004).
11. M. Ip, S.L. Lui, V.K.M. Poon, I. Lung and A. Burd, J. Med. Microbiol., 1, 59 (2006).
12. H.J. Klasen, Burns., 2, 117 (2000).
13. R. Sarkar, P. Kumabhakar and A.K. Mitra, Digest. J. Nanomater. Biostruct., 2, 491 (2010).
14. M. Rai, A. Yadav and A. Gade, Biotechnol. Adv.,1, 6 (2009).
15. J.L. Elechiguerra, J.L. Burt, J.R. Morones, A. Camacho-Bragado, X. Gao, H.H. Lara and M.J. Yacaman, J. Nanobiotechnology., 6, 6 **(**2005).
16. N. Sap-Iam, C. Homklinchan, R. Larpudomlert, W. Warisnoicharoen, A. Sereemaspun and S.T. Dubas, J. Appl. Sci., 3132 (2010).
17. C. Marambio-Jones and E.M.V. Hoek, J. Nanoport.Res., 5, 1531 (2010).

18. A.M.D. Farooqui, P.S. Chauhan, P.K. Moorty and J. Skark, Digest. J. Nanomater. Biostruct., 43, (2010).
19. S. Shrivastava, T. Bera, A. Roy, G. Singh, Ramachandrarao and D. Dash, Nanotechnol., 22, 225103 (2007).
20. A. Sillikaite, I. Prosycevas, J. Puiso, A. Juraitis and A. Guobiene, Mate. Sci., 4, 287 (2006).
21. Fu Mouxing, Li Qingbiao, Sun Daohua, Lu Yinghua, He Ning, Deng Xu, Wang Huixuan and Huang Jiale, Chinese J. Chem. Eng., 1, 114 (2006).
22. M. Linga Rao and N. Savithramma, J. Pharm. Sci. Res., 3, 1117 (2011).
23. Q.L. Feng, J. Wu, G.Q. Chen, F.Z. Cui, T.M. Kim, J.O. Kim, J. Biomed.Mater. Res., 4, 662 (2000).
24. J.A. Spadaro, T.J. Benger, S.D. Barranco, S.E. Chapin and R.O. Becker, Antimicrob. Agents. Ch., 5, 637 (1974).
25. P.D. Bragg and D.J. Rannie, Can. J. Microbiol., 6, 883 (1974).
26. K.I. Bataresh, J. Antimicrob.Chemoth., 2, 546 (2004).
27. R.M. Richards, H.A. Odelola and B. Anderson, Microbes., 157-158, 151, (1984)
28. NCCLS. Performance Standards for antimicrobial susceptibility testing. 1^{2th} Informational supplement M100-S12. National Committee for Clinical Laboratory Standards. Villanova, PA. 2002.
29. A.M. Fayza, K. Balaji, M. Girilal, R. Yadav, P.T. Kalaichelvam and R. Venketasan, Nanomedicine., 1, 103 (2010).

****