The Pharma Innovation Journal 2023; 12(3): 1158-1162

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(2): 1158-1162

www.thepharmajournal.com
Received: 16-12-2022
Accepted: 27-01-2023

T Sathyabama
Assistant Professor, Department of Veterinary Physiology, VC&RI, Namakkal, Tamil Nadu, India

A Kirubakaran
VUTRC, Erode, Tamil Nadu, India

P Selvaraj
Dept. of Veterinary Physiology, VC&RI, Namakkal, Tamil Nadu, India

Corresponding Author:
T Sathyabama
Assistant Professor, Department of Veterinary Physiology, VC&RI, Namakkal, Tamil Nadu, India

Synthesis, characterization and evaluation of bactericidal activity of silver nano particle

T Sathyabama, A Kirubakaran and P Selvaraj

Abstract
The current study was undertaken to explore the effect of silver nano particle as a topical bactericidal agent. Silver nano particle (AgNP) was synthesized by chemical reduction method and characterized by Transmission Electron Microscope (TEM). The bactericidal activity of AgNP was determined using the disc, gauze and well diffusion method against common clinical pathogenic bacteria. The highest bactericidal effect was observed at the concentration of 40 µL as a maximum diameter of zone of inhibition (mm) and the observed results showed that the AgNPs have potent bactericidal activity against both gram positive and gram negative bacteria. The present study indicated that AgNP can effectively be used as an alternate to antibiotic topical agent, when antibiotic resistance is suspected.

Keywords: Silver nano particle, synthesis, characterization, pathogenic bacteria, bactericidal activity

Introduction
Among the most commonly used topical antimicrobial agent, silver based topical application is employed due to its broad spectrum activity, efficacy and lower costs with low toxicity. Increased therapeutic use of antibiotics resulted in the emergence of antibiotic resistant bacteria especially multidrug resistant (MDR) bacteria, a major continuous threat to treatment. Silver, and its derivatives, are known strong effective agent against bacteria, fungi and viruses (Lok, *et al*., 2006) [12]. To curtail the development of antibiotic resistance, various nano metal preparations like silver, gold, copper, zinc have been incorporated in many pharmaceutical preparations for their catalytic, optical, electronic, magnetic and anti-microbial properties (Cao, 2004 and Cho, 2005) [3, 4]. Several previous studies have shown that silver nanoparticles (Ag-NPs) are effectively incorporated into wound and burn dressings (Jia, *et al*., 2007) [10]. Gemmell *et al*., 2006 reported that Ag-NPs were effective against Pseudomonas aeruginosa, ampicillin resistant Escherichia coli, erythromycin-resistant Strepococcus pyogenes, methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Staphylococcus aureus (VRSA). Though various methods are employed for the synthesis of AgNPs (Gudikandula and Maringanti, 2016) [8], chemical method proved to be nontoxic and cost-effective. In the current study, a chemical reduction method is used for the synthesis of AgNps and is evaluated for the bactericidal activity after morphological characterization.

Materials and Methods
The chemical reagents - Silver nitrate and tri sodium citrate were purchased from Sigma-Aldrich and all the reagents required for the study were prepared in double distilled water.

1. Chemical Synthesis of Silver Nanoparticles
The silver nano particle was prepared by chemical reduction method as per Fang *et al*., (2005) [5]. A stock aqueous solution of 1mM $AgNO_3$ and 1% tri sodium citrate solution were prepared. A working solution of 500 ml of $AgNO_3$ was heated to boiling. To this solution, 10 ml of trisodium citrate was added drop by drop with vigorous magnetic stirring continuously for 30min. and heated until noticeable colour change. This indicates the conversion of $AgNO_3$ into AgNP. Green–gray silver nano particle were obtained immediately after cooling the solution.

2. Characterization of Silver Nanoparticles
The identity and size of obtained Ag-NPs was confirmed by ultraviolet–visible spectrophotometer (UV–Vis Spectrophotometer, PerkinElmer LAMBDA, Waltham, USA) and characterized by transmission electron microscopy (TEM).

3. Bactericidal activities of Silver nanoparticles

The bacterial strains *viz.*, gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria were obtained from multidrug-resistant (MDR) clinical pathogenic isolates and passaged three times in nutrient broth medium to optimize their physiological activities.The bactericidal activity studies were performed by three methods as follows in triplicate.

1. Paper disc diffusion method

Using disc diffusion method, the bactericidal activity of AgNP was determined. The bacterial strains *viz.*, gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) were tested against the synthesized AgNP. A range of concentrations of AgNP were prepared (10, 20, 30, 40 and 50 µL) and paper discs (5 mm) were soaked in each concentration. Using a glass rod, the bacterial strain (100 mL) was inoculated by smearing on the medium and dried for 5 min. The synthesized AgNP loaded discs were placed on the surface of the medium and allowed to diffuse for 5 min. The petridish were incubated at 37° C for 24 h and were then examined for the presence of zones of inhibition (mm). A disc soaked in sterilized water (20µL) was used as a negative control and silver nitrate ($AgNO_3$) disc (20 µL and 40 µL /disc) was used as a positive control. After deducting the diameter of the positive control zones from the AgNP zone and the obtained zone of inhibition diameter, expressed in millimeter unit (mm) was tabulated.

2. Agar well-diffusion method

Briefly, 20 mL of nutrient agar medium was poured into sterilized Petri dishes. To prepare the bacterial colonies (Gram-positive and Gram-negative bacteria) of 1 x 10^5 CFU/mL one day old bacterial cultures was used. Agar wells (8 mm diameter) were prepared with the help of a sterilized template. The wells were injected with a range of 5, 10, 20, 30, 40 and 50 µL concentrations of synthesized AgNP solution. The plates were further incubated at 37° C for 24 h and examined for the presence of zones of inhibition and measured (mm).

3. Gauze diffusion method

After confirming the antimicrobial potential of AgNP using disc and well diffusion methods, the effective dose was incorporated into a gauze cloth. As detailed above, instead of paper disc, sterile surgical gauze of 5 mm X 5mm size was soaked in 40 µL synthesized AgNP solution, 40 µL of 1 mM $AgNO_3$ as a positive control, 40 µL of double distilled water used in the experiment as a negative control along with a sterile surgical gauze as a blank. Aliquots of bacterial culture (100 mL) were spread on petri dishes containing agar-solidified Luria broth (LB) medium. The prepared and loaded gauze as specified above, were placed and incubated at 37° C for 24 h. The presence of zones of inhibition (mm) was recorded.

Results and Discussion

1. Chemical synthesis of silver nanoparticles

The silver nanoparticles are formed by the chemical reduction of $AgNO_3$, with a powerful reducing and stabilizing agent – Tri sodium citrate. The formed AgNP colloids are affected by initial concentration of $AgNO_3$ (molar ratio) and stabilizer concentration. Among the various methods available for the synthesis of silver nanoparticles, the best and most easy economical high yielding method without aggregation is chemical reduction method (Iravani *et al*., 2014) [9]. Green–gray silver nano particle were obtained immediately after cooling the solution (Fig. 1).

2. Characterization of Silver Nanoparticles

The synthesized Ag-NP colloids were analyzed for absorbance at 300–700 nm in a UV-visible (UV-Vis) spectrophotometer. The identity of synthesized Ag-NP colloids by UV– visible spectrophotometer absorption spectra had a peak value of 420 nm (Fig. 2).

Earlier reports of optical measurements of Ag-NP confirmed by UV-Vis spectrophotometer analysis showed an absorbance peak at 420 nm which was specific for silver nanoparticles (Gudikandula and Maringanti, 2016) [8] whereas Sayed *et al*. (2015) [17] reported the biosynthesis of Ag nanoparticles using *Aspergillus sp*. in the range of 525 nm.

Stable and mono dispersed AgNP synthesized chemically were recovered and characterized by transmission electron microscopy (Fig. 3). The identity and size of synthesized AgNP were spherical and are in a range of 43.82 to 73.24 nm.

The resultant TEM images are in agreement with the reports of Panacek *et al*. (2006) [14], Anil Kumar *et al*. (2007) [2] and Galdiero *et al*. (2011) [6] who showed spherical shaped chemically synthesized silver nanoparticles in a range of 10 to 30 nm while Abalkhila *et al*., 2017 [1] recorded 10-60 nm spherical sized AgNP using extracts from *A. vera*, *P. oleracea* and *C. dactylon*. Similarly, Iravani *et al*., 2014 [9] reported chemically synthesized AgNP with tri sodium citrate as reducing cum stabilizing agent yield a nano particle size of 30-60 nm.

The synthesis of Ag-NP and characterization of Ag-NP by TEM and UV–visible spectrophotometer revealed spherical nanoparticles exhibiting a range of 40-75 nm sizes at 405-420 nm.

3. Bactericidal activity studies of Silver nanoparticles

Results from antibacterial activity assessment of AgNP as diameter of inhibition zones (mm) are presented in Table 1 A, B, C and depicted in Fig.4 A, B, C.

Evaluation of the bactericidal activities of Ag-NP revealed that the inhibition zones (mm) around Ag-NPs-loaded discs found to have potent bactericidal activities against both Gram positive and Gram negative bacteria. The highest effect was observed at a concentration of 40 µL in disc and gauze diffusion studies. In well diffusion method both 40 µL and 50 µL concentration range gave almost same diameter of zone of inhibition. The diameter of the inhibition zone was smaller at a concentration of 5.0 µL. These results are similar to those reported by other investigators (Abalkhila *et al*., 2017**,** Morones *et al*., 2005 and Kim *et al*., 2007) [1, 13, 11].

Previous studies of Sondi *et al*. (2004) [18], Sarkar *et al*. (2007) [16] and Sayed *et al*. (2015) [17] indicated that the synthesized nonoparticles using *aloe vera* and *E. coli* have antimicrobial activity against a wide range of microorganisms.

Summary

The chemically synthesized of Ag-NP was found to have potent bactericidal activities against both Gram positive and Gram negative bacteria. The results clearly indicate that silver nanoparticles could provide a safer alternative to conventional antimicrobial agents in the form of a topical pharmaceutical formulation.

Conclusion

The present study provides an evidence for an easy and cheap method for synthesizing potent bactericidal Ag-NPs and demonstrates their effectiveness against common pathogenic bacteria as an alternative approach to antibiotics, when antibiotic resistance is suspected.

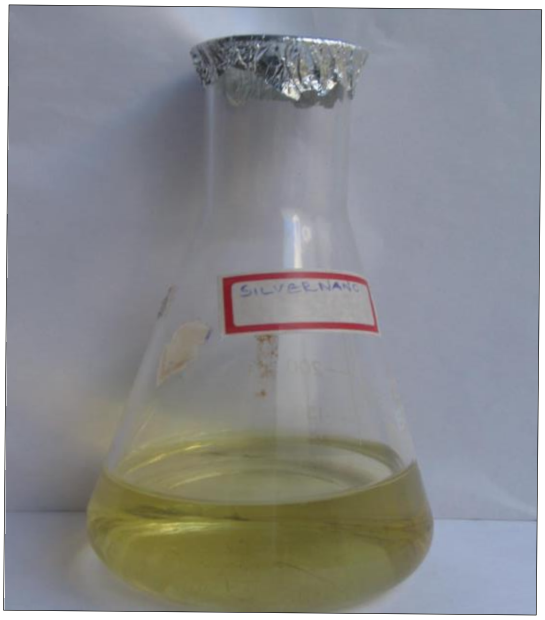


Fig 1: Chemical synthesis of AgNPs

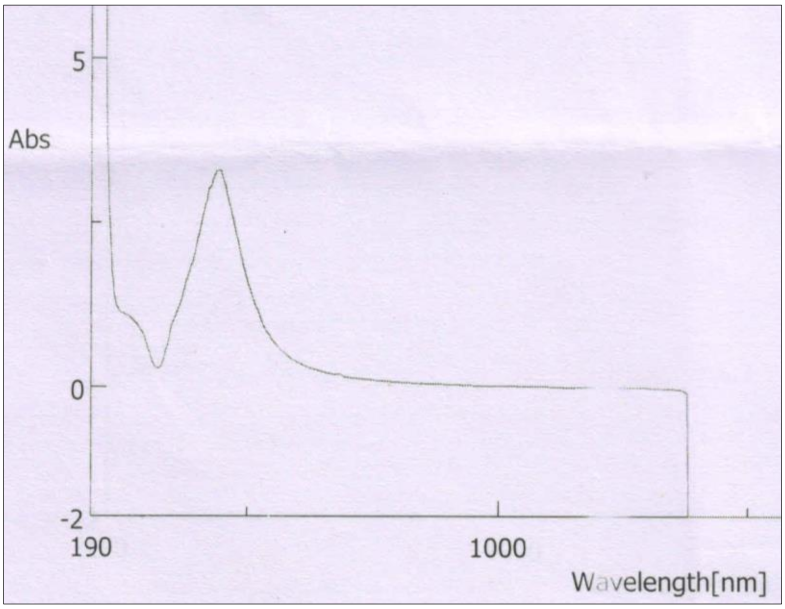


Fig 2: UV-Vis Spectrophotometer analysis of chemically synthesized AgNPs

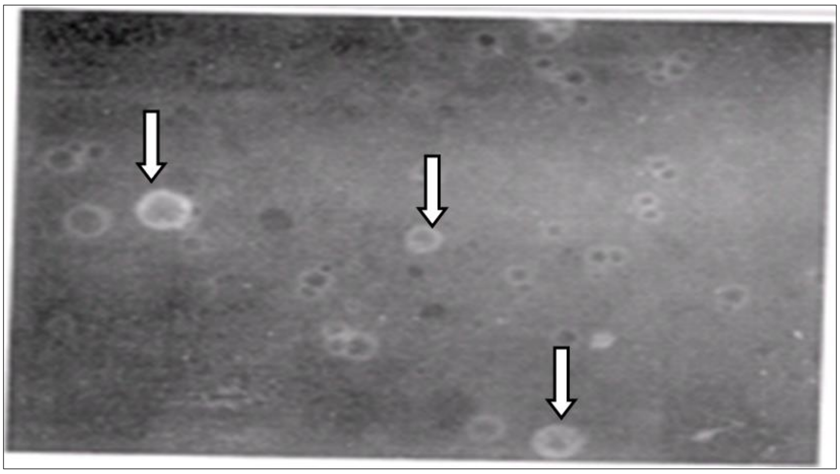


Fig 3: TEM images of chemically synthesized AgNPs.

Table 1: Antibacterial activity of chemically synthesized Ag-NPs
A. Disc diffusion method

Zone of inhibition (mm diameter)								
Bacterial strain	Concentration							
	AgNP(µl)					Silver nitrate ($AgNO_3$)		Sterile water (Negative control) 20µl
	10	20	30	40	50	20µl	40µl	
Gram-positive (*Staphylococcus aureus*)	13.1	12.9	15.4	18.2	17.7	14.2 (C_1)	17.4 (D)	0
Gram-negative (*Escherichia coli*)	3.1	3.9	4.7	5.8	5.2	2.7 (D)	4.4 (C_1)	0

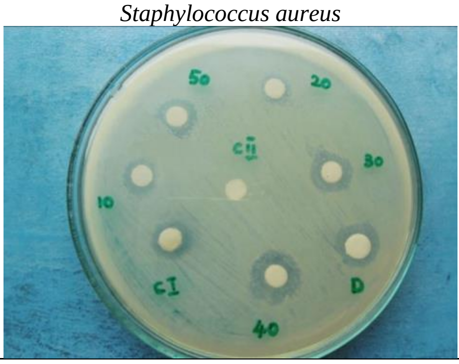
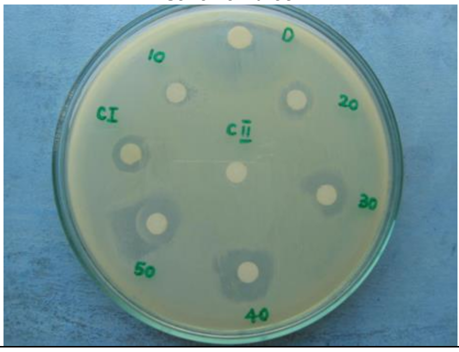
B. Well diffusion method

Zone of inhibition (mm diameter)						
Bacterial strain	Concentration of AgNP (µl)					
	5	10	20	30	40	50
Gram-positive (*Staphylococcus aureus*)	0	12.4	12.4	12.6	13.6	13.6
Gram-negative (*Escherichia coli*)	0	2.8	3.3	3.5	4.3	4.2

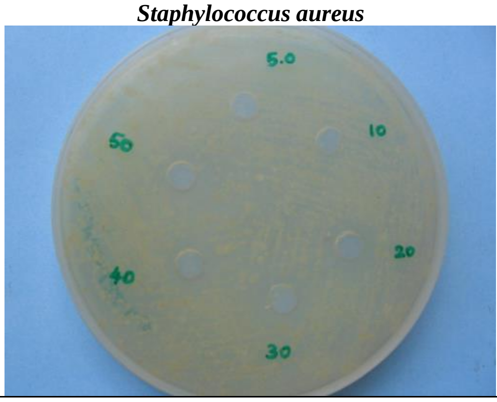
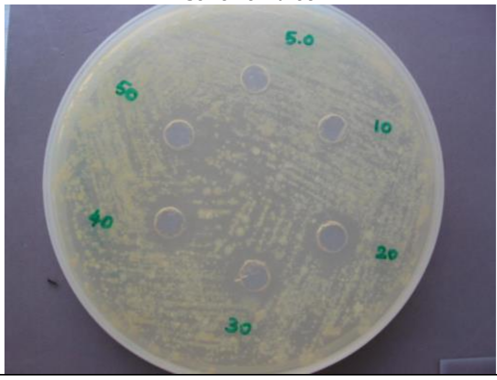
C. Gauze diffusion method

Zone of inhibition (mm diameter)				
Bacterial strain	**Blank (sterilegauze) (CIII)**	**Sterile water (Negative control) 40µL (CI)**	**Silver nitrate ($AgNO_3$)- 40µL (Positive control) (CII)**	**Silver Nanoparticle (AgNP) (40µL) (SII)**
Gram-positive (*Staphylococcus aureus*)	0	0	3.8	4.3
Gram-negative (*Escherichia coli*)	0	0	0.7	2.7

A. Disc diffusion method

A.	*Staphylococcus aureus* 	C II – Negative control (20 µl sterile water) C I – Positive control (20 µl $AgNO_3$) D – Positive control (40 µl $AgNO_3$)
B.	*Escherichia coli* 	C II – Negative control (20 µl sterile water) C I – Positive control (40 µl $AgNO_3$) D – Positive control (20 µl $AgNO_3$)

B. Well diffusion method

A.	***Staphylococcus aureus*** 	Concentration of AgNP (µl) Range - 5,10,20,30,40,50
B.	***Escherichia coli*** 	Concentration of AgNP (µl) Range - 5,10,20,30,40,50

c. Gauze diffusion method

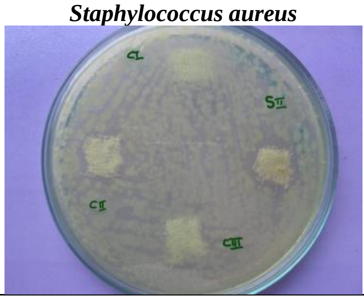
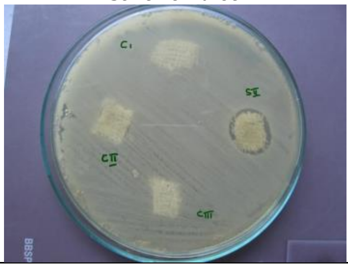
A.	***Staphylococcus aureus*** 	C I – Negative control (40 µl sterile water) C II – Positive control (40 µl $AgNO_3$) C III – Blank (sterile gauze) S II – AgNP (40 µl $AgNO_3$)
B.	***Escherichia coli*** 	C I – Negative control (40 µl sterile water) C II – Positive control (40 µl $AgNO_3$) C III – Blank (sterile gauze) S II – AgNP (40 µl $AgNO_3$)

Fig 4: Antibacterial activity of chemically synthesized Ag-NPs

References

1. Abalkhila TA, Alharbia SA, Salmena SH, Wainwright M. Bactericidal activity of biosynthesized silver nanoparticles against human pathogenic bacteria. Biotechnology & Biotechnological Equipment. 2017;31:411-417.
2. Anil Kumar S, Abyaneh MK, Gosavi SW, Kulkarni SK, Pasricha R, Ahmad A, *et al*. Nitrate reductase mediated synthesis of silver nanoparticles from $AgNO_3$. Biotechnology Letters. 2007;29:439-445.
3. Cao G. Synthesis, Properties and Applications. In: Nanostructures and nanomaterials. London. Imperial College Press, 2004.
4. Cho KH, Park JE, Osaka T, Park SG. The study of antimicrobial activity and preservative effects of nanosilver ingredient. Electrochim Acta. 2005;51:956-960.
5. Fang J, Zhang C, Mu R. The study of deposited silver particulate films by simple method for efficient SERS. Chemical Physics Letters. 2005;401:271-275.
6. Galdiero S, Falanga A, Vitiello M, Cantisani M, Marra V, Galdiero M. Silver nanoparticles as potential antiviral agents. Molecules. 2011;16:8894-8918.
7. Gemmell CG, Edwards DI, Fraise AP. Guidelines for the prophylaxis and treatment of methicillin-resistant Staphylococcus aureus (MRSA) infections in the UK. Journal of Antimicrobial Chemotherapy. 2006;57:589-608.
8. Gudikandula K, Maringanti SC. Synthesis of silver nanoparticles by chemical and biological methods and their antimicrobial properties. Journal of Experimental Nanoscience. 2016;11:714-721.
9. Iravani S, Korbekandi H, Mirmohammadi SV, Zolfaghar B. Synthesis of silver nanoparticles: chemical, physical and biological methods. Research in Pharmaceutical Sciences. 2014;9(6):385-406.
10. Jia J, Duan YY, Wang SH, Zhang SF, Wang ZY. Preparation and characterization of antibacterial silver-containing nanofibers for wound dressing applications. Journal of US- China Medical Science. 2007;4(2):52-54.
11. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee H, *et al*. Antimicrobial effects of silver nanoparticles. Nanomedicine. 2007;3(1):95-101.
12. Lok CN, Ho CM, Chen R, He QY, Yu WY, Sun H, *et al*. Proteomic analysis of the mode of antibacterial action of silver nanoparticles. Journal of Proteome Research. 2006;5(4):916-924.
13. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramírez JT, *et al.* The bactericidal effect of silver nanoparticles. Nanotechnology. 2005;16(10):2346-2353.
14. Panacek A, Kvitek L, Prucek R, Kolář M, Večeřová R, Pizúrová N, *et al.* Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity. Journal of Physical Chemistry. 2006;110(33):16248-16253.
15. Sargowo D, Handaya AY, Widodo A, Lyrawati D, Tjokroprawiro A. Aloe Gel Enhances Angiogenesis. The Indonesian Biomedical Journal. 2011;3:204-215.
16. Sarkar S, Jana AD, Samanta SK, Mostafa G. Facile synthesis of silver nano particles with highly efficient anti-microbial property. Polyhedron. 2007;26(15):4419-4426.
17. Sayed SR, Bahkali AH, Bakri MM, Hirad AH, Elgorban AM, El-Metwally MA. Antibacterial activity of biogenic silver nanoparticles produced by Aspergillus terreus. International Journal of Pharmacology. 2015;11(7):858-863.
18. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on E. *coli* as a model for Gram-negative bacteria. Current Opinion in Colloid & Interface Science. 2004;275;177-182.