

Silver Nanoparticle: A Bactericidal Agent for Pathogenic Poultry Bacteria

Geetha Kathiresan, Kanimozhi, N. Arulnathan

Abstract: The biocompatibility and strong antimicrobial ability of silver and its derivatives have attracted the biomedical researchers for biomedical implants, water filtration and other bio-applications. Recently silver nanoparticles have also been engineered for biological applications, specifically as antimicrobial agents to overcome the antibiotic resistance in animals and humans. In this context, we have evaluated the antimicrobial activity of silver nanoparticles against the pathogenic poultry strains that affect the poultry production and performance. Silver nanoparticles were synthesized by oxidation reduction method using silver nitrate solution with sodium borohydride as the reducer. The particle size ranging from 168.57nm – 425.41nm was revealed from the SEM micrograph. The absorption wavelength of the UV visible spectroscopy at 392 nm ensured the presence of silver nanoparticles in the sample. The diffraction peak derived from 38° (111) indicates the presence of Face Centered Cubic pure crystalline silver. Antibiotic Disc Diffusion Test (ADDT), microtiter assay and ex-vivo studies revealed the effective antimicrobial activity, biofilm inhibition effect and also increased egg hatch rate than control group.

Index Terms: Antimicrobial; Biofilm inhibition; Ex-vivo; Oxidation reduction method; Pathogenic poultry strains; Silver nanoparticles;

I. INTRODUCTION

Antimicrobial activity of the material is very essential in the application of medicine, food packaging, textiles, water purification and also disinfectants. The conventional inorganic antibiotics and organic materials are more toxic, but is used to prevent the side effects and also provide better bacteriostatic and bactericidal effects (Das et al., 2011). Antibacterial activity of silver is preferred for therapeutic agent especially for infectious disease (Lara et al., 2011). Other than silver, silver precursors are also used as the antimicrobial agent for example silver nitrate is used for the *Aphthous stomatitis* (Sondi and Sondi, 2004) and Silver sulfadiazine is used to heal the burn wounds (Alexander, 2009).

Nano sized silver particle is more preferable than the other form of silver precursors like silver nitrate, silver oxide, etc. The enhanced surface area of the nano sized silver particles provides the more toxic effect to the microbes (Prabhu and Poulouse, 2012; Kim et al., 2007). The bacteriostatic effects of silver nanoparticles were proven against *Staphylococcus*, *Bacillus*, *Staphylococcus aureus* and *Pseudomona aereginosa* (Prabhu et al., 2013). Surface coated amphiphilic hyper branched silver nanoparticle has exhibited the toxic effect to pseudomonas (Reidy et al., 2013) and also inhibited Methicillin resistant *Staphylococcus aureus* growth.

Revised Manuscript Received on December 22, 2018.

Geetha Kathiresan, Nanotechnology Division, Periyar Maniammai Institute of Science & Technology, Thanjavur, Tamil Nadu, India

Kanimozhi, Nanotechnology Division, Periyar Maniammai Institute of Science & Technology, Thanjavur, Tamil Nadu, India.

N.Arulnathan, Veterinary college and Research Institute, Tirunelveli, India

The mechanism behind this antibacterial activity of silver is studied by different ways. The Reactive Oxygen Species (ROS) released by the silver nanoparticle has been preventing the DNA replication, uncontrolled oxidation of proteins, breakdown of membrane function (Reidy et al., 2013) and interaction with sulfur and phosphorus will cause the problems in DNA replication (degradation of bacterial DNA with *E. coli* is recently studied (Reidy et al., 2013; Percival et al., 2005). Damages of phospholipid membrane induce the uptake of particles for functioning as deposits to release the silver ions (Reidy et al., 2013; Hajipour et al., 2012). The sustained delivery of silver nanoparticles was achieved by incorporation of particle into the matrix or encapsulation by the polymers. These materials are evaluated as a good microbicides with small doses, minimal toxicity and side effects (Lara et al., 2010). Wet chemical methods using stabilizers like PVA, PVP and sucrose is more preferred for silver nanoparticle synthesis (Zhang et al., 2001) than other methods.

The productivity of poultry industry is challenging especially against the microbial diseases. Even if it is recovered by synthetic antibiotics, long term administration leads to antibiotic resistant trait in those particular strains. The recent nano medicine development has been able to provide the metal and metal oxide nanoparticle Al_2O_3 , Fe_3O_4 , CeO_2 , ZrO_2 , and MgO . However, studies reveal that the silver nanoparticles in poultry sector are too limited (Sawosz, 2007; Grodzik and Sawosz, 2006).

When it is administered at pre hatching condition, by reducing the microbial population, the silver nanoparticle will enhance the health and immune status of the chick (Prabu et al., 2013). The present study is focused on antimicrobial effect of synthesized colloidal silver nanoparticles against selected poultry pathogenic bacterial strains and evaluation of silver nanoparticles on hatch rate, hatch weight and growth performance by ex-vivo administration.

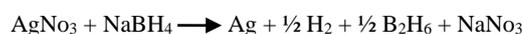
II. MATERIALS AND METHODS

A. Material

The analytical grade of Silver Nitrate ($AgNO_3$) were obtained from Sigma-Aldrich and sodium borohydride ($NaBH_4$) is purchased from NICE, Cochin. The bacterial strains *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella pneumoniae* and nutrient broth medium for antibacterial Disc Diffusion Test (DDT) were collected from the department of Biotechnology, Periyar Maniammai University, Thanjavur.

B. Synthesis of silver nanoparticles

Oxidation reduction method was used for silver nanoparticle synthesis. 0.001 M of silver nitrate solution is produced using double distilled water and 0.010 M of sodium borohydride was added drop by drop in to the 0.001 M of silver nitrate solution under magnetic stirring.



The solution was mixed vigorously until the color changed in the order of pale



Silver Nanoparticle: A Bactericidal Agent for Pathogenic Poultry Bacteria

yellow, red wine and very dark brown which is almost nearest to black color. The color changes during synthesis were shown in Figure 1. Transparent yellow solution indicates the reduced silver ions from the precursor to form the silver nanoparticles. Final dark color exposed in the image indicates the stabilized silver nanoparticle formation and it will be maintained up to several months. Upon stirring, the aqueous solution was centrifuged at 1500 rpm at 30 min. The precipitate was collected and dried at 100°C. The dark final ash colored powder was stored in an Eppendorf for further characterization and applications.



Figure 1: Indication of color changes during silver nanoparticle synthesis

C. Antibacterial Disk Diffusion Test (DDT)

1. Bacterial strains

Four bacterial strains namely *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Klebsiella pneumoniae* were taken for the study. These strains were sub cultured on high media agar and maintained for experiments.

2. Disc Diffusion test

Solutions of three different concentrations of silver nanoparticle were tested for antibacterial activity. The preferred concentration 10, 20, 30 $\mu\text{g/ml}$ of silver nanoparticle were prepared and ultrasonicated to obtain a homogenous solution. The inoculums of the sub cultured bacterial strains were spread and plated in the nutrient agar medium under sterile condition. The Whatman no. 1 filter papers were cut into 1 cm^2 small pieces and sterilized. The sterilized discs were dipped into the different concentration of the test samples and placed in the petri dishes. Finally the plates were incubated for twenty four hours at 37°C. The zone around the film was measured on the subsequent day using a centimeter scale.

3. Planktonic growth inhibition effect

The assay was conducted by sterile dispensing of 20 μl of bacteria onto microtiter plates along with nanoparticles of the given concentration. Luria Bertani broth (180 μl) served as media. Microliter wells without nanoparticles were taken as controls. Four rows of 48 wells were used for each strain. Each plate included 24 wells as controls and 12 wells as blank. Each concentration of silver nanoparticles was prepared in duplicate. The quantitative analysis of antimicrobial activity was measured using a microtiter plate reader at a wavelength of 600 nm. The average absorbance of the control wells were subtracted from the test wells. Growth inhibition was assessed by plotting the graph for time versus O.D.

4. Biofilm assay using microtiter plate

Four rows of 48 wells were used for each strain. Each plate included 24 wells as controls and 12 wells as blank. Each concentration of silver nanoparticles was prepared in duplicate. After 24 hour incubation, the medium was removed and the plates were washed three times with distilled water to remove loosely

attached bacteria. Plates were air dried for 45 min prior to staining, then each well was loaded with 200 μl of 0.2% of crystal violet stain in methanol for 30 min. The stained plates were rinsed with distilled water until no stain was visible. The quantitative analysis of the biofilm production was performed by adding 200 μl of 96% ethanol to de-stained wells. The absorbance (A) of the crystal violet present in the de-staining solution was measured using a microtiter plate reader at a wavelength of 570 nm. The average absorbance from control wells were subtracted from the test wells. The same experiment was repeated after 6 and 12 hours of incubation.

5. Ex-vivo Studies

Four groups of 30 fertile eggs laid by Namakkal Black chicken in our University farm were selected and weighed for incubation. First group (30 eggs) was used as a control and another three groups of eggs were inoculated with silver nanoparticle (10, 20 and 30 $\mu\text{g/kg}$) using micro needle at the allantoic cavity and the injected hole was sterilized and closed with alcohol swabs. And finally all the groups were incubated with 38°C, 50% humidity with 6 turning/24 hours. At day 19th onwards the humidity was increased up to $55 \pm 5^\circ\text{C}$ and the temperature was reduced up to 36°C. 21st day onwards the hatched chicks were collected, weighed and physical characteristics were observed.

III. RESULT AND DISCUSSION

A. Optical property analysis Using UV Visible Spectroscopy:

Ultraviolet-visible analysis was done by using SYSTRONICS PC Based Double beam spectrophotometer 2202 with resolution of 1 nm between 300 to 600 nm. Reduction of AgNO_3 to Ag^+ was confirmed by color change from colorless to brown. Formation of silver nanoparticles can be easily detected by measuring the optical density of solutions/suspensions. The UV Visible spectrums shown in Figure 2 express the absorption band at 392 which confirms the presence of silver nanoparticles in the solution. The broadening of resonance band indicates the presence of nanoparticle with the reference of quantum size theories (Rai et al., 2009).

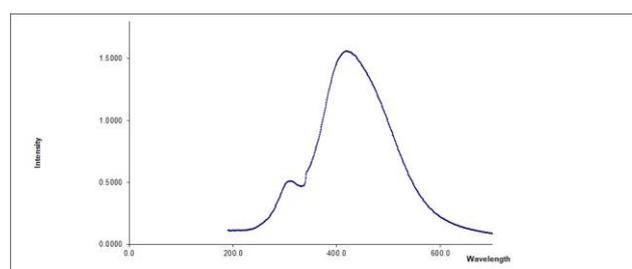


Figure 2: UV Spectrum of silver nanoparticles

B. XRD:

The resulting diffraction peaks obtained from powdered form of silver nanoparticle is shown in Figure 3. The diffraction peak derived from 2θ value 38 (Lattice plane 111) indicates the presence of Face Centered Cubic pure crystalline silver. And the additional peaks attained at 43.96 (220), 64.50 (311) and 76.52 (222) also correspond to the silver nanoparticles. The spectrum was compared with JCPDS data and the result revealed that cubical silver nanoparticles were present in the sample. Our results are comparable with the study done by Soleimani and Habibi (2017) which shows similar evaluation of silver nanoparticles biosynthesis.



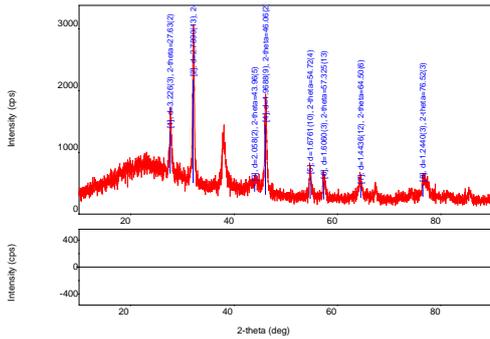


Figure 3: XRD of Silver nanoparticles

C. Particle size and shape analysis using SEM analysis:

Scanning Electron Microscopic (SEM) analysis was imaged using model VEGA 3 LMU made by TESCAN. A very small drop of silver nanoparticle solution was dried over the cover slip in hot air oven. As it was a conductive smear it was imaged directly by the SEM without conductive coating. The SEM images shown in Figure 4 are taken from the sample, which is fabricated without any stabilizer. More particle agglomeration was noticed. Hence, the specific size and shape of the silver nanoparticle is not determined. In order to prevent the agglomeration, the solution was diluted then dropped and smeared for SEM analysis. It is revealed that the sphere shaped silver nanoparticle were present in the size range of 168.57 nm – 425.41 nm. The result is supported by the study done by Kota et al., (2017) which shows biosynthesis of silver nanoparticles from plant origin.

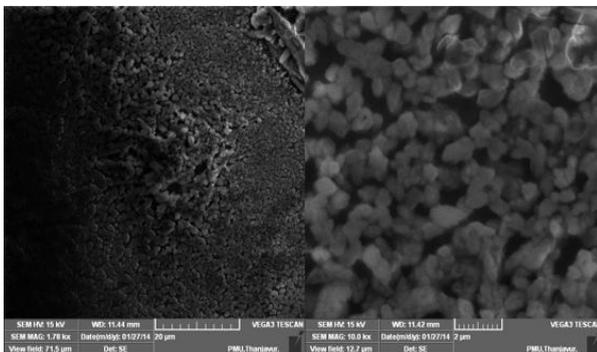


Figure 4: SEM images of silver nanoparticles

D. Antibacterial Disc Diffusion Test (DDT):

The inhibitory action of the synthesized silver nanoparticles was depicted in Figure 5. As the concentration level increases, the zone of inhibition also increases, which implies that the bacterial growth suppression rate increases. The result of this study clearly demonstrates that, the colloidal silver nanoparticle inhibits the growth and multiplication of selected bacterial strains. Silver nanoparticles expressed high inhibitory effect against *Streptococcus pyogenes*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Escherichia coli* at 30 µg/ml. Silver nanoparticles showed strong inhibitory effect (2 cm) against *Klebsiella pneumonia* at low concentration (10 µg/ml). The inhibitory effect of all the discs was not uniform in all the direction, which might be due to the diffusivity of silver nanoparticles solution from the disc (Kashid et al., 2017).

Table 1: Inhibitory zone level of silver nanoparticles against four different bacterial strains at different concentrations.

No.	Bacterial pathogens	Zone of inhibition in cm		
		10 µl/ml	20 µl/n	30 µl/m
1	<i>Staphylococcus aureus</i>	0.5	1.3	1.8
2	<i>Escherichia coli</i>	1.5	2.4	3.8
3	<i>Klebsiella pneumoniae</i>	0.8	1.0	1.4
4	<i>Streptococcus pyogen</i>	1.2	1.7	3.4

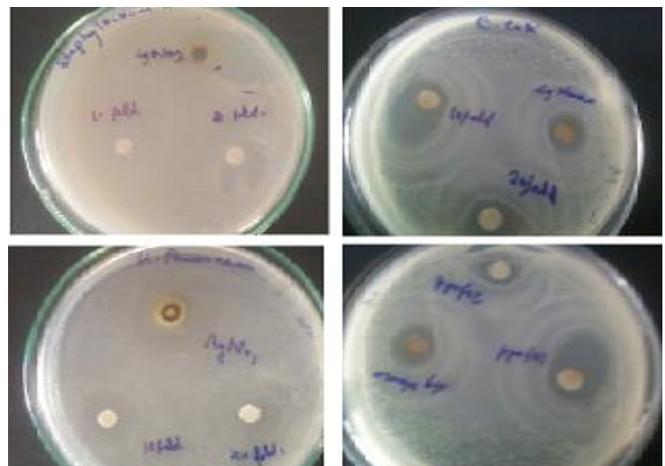


Figure 5: Antibacterial effect of silver nanoparticles by Disc Diffusion Method

Although the mechanism behind the antibacterial effect of silver is unknown, there are various theories on the action of the silver nanoparticles on the bacteria. By forming pits on the surface of the cell, silver nanoparticles are accumulated on bacterial surface. This is due to the anchoring of silver onto bacterial cell wall which leads to death of the bacteria (Sondi and Sondi, 2004; Prabhu and Poulouse, 2012). SEM images of both treated and non-treated *E.coli* with silver nanoparticles are shown in Figure 6. The left side of the image expresses the healthy bacterial colony with good morphological structure and the right one shows the structurally changed, size reduced and killed bacterial colonies by silver nanoparticle.

Silver nanoparticles release silver ions which plays a major role in the interaction of bacteria. The catalytic activity of the silver ions induced to form the disulfide bonds (R-S-S-R) to change the protein structure. It leads to structural changes in cellular enzyme and affects their function by removing free sulfhydryl groups (Harrison, 2006). So inactivation of key enzymes occurs and affects cellular respiration (Jeevan, 2012) and denaturing of bacterial protein leads to cell death (Karnib, 2013; Selvakumar, 2013). It also causes an interruption in the DNA replication, which further stops the growth of bacteria (Carlson, 2008). So, the results reflects that the silver nanoparticle treated bacteria were killed due to bactericidal activity.



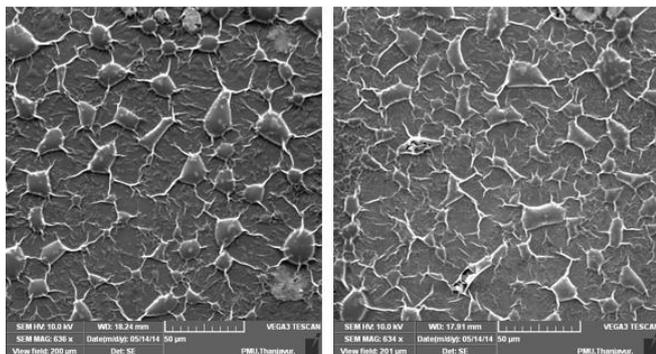


Figure 6: SEM image of *E. coli* and silver nanoparticles treated *E. coli*

E. Planktonic growth and biofilm inhibition of silver nanoparticles

The planktonic growth in presence of silver nanoparticles with different concentration (10-30 $\mu\text{g/ml}$) has been carried out and the comparison of planktonic growth for *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes* and *Klebsiella pneumoniae* is schematically shown in Figure 7. *Staphylococcus aureus*, *Escherichia coli* showed maximum inhibition compared to *Klebsiella pneumoniae* and *Streptococcus pyogenes*.

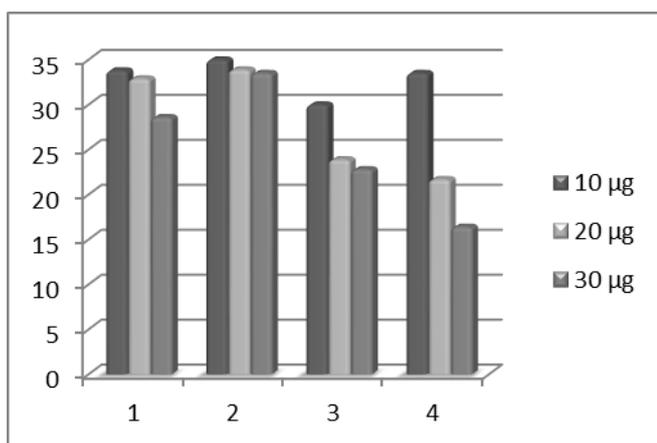


Figure 7: Comparison of planktonic growth in presence of silver nanoparticles.

The biofilm formation of pathogenic poultry microbes was evaluated with three different concentrations of silver nanoparticles using microtiter plate reader at 570 nm. The result shown in Figure 8 reveals that the biofilm inhibitory effect was achieved and also the inhibition rate was increased when the concentration of silver nanoparticles was increased. The inhibition rate of biofilms *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Streptococcus pyogenes* are discussed below for 6, 12 and 24 hours of incubation of biofilms. *Staphylococcus aureus* and *Escherichia coli* showed maximum inhibition compared to *Klebsiella pneumoniae* and *Streptococcus pyogenes*.

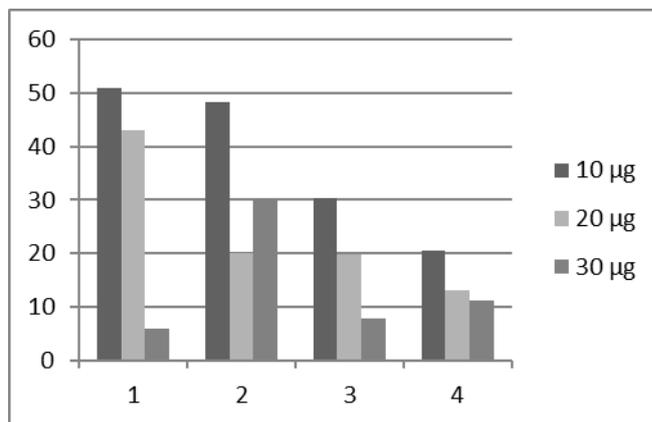


Figure 8: Comparison of biofilm inhibition

The minimum inhibitory concentration for both biofilm and planktonic growth is found to be 10 $\mu\text{g/ml}$. The percentage of inhibition was found to be around 30% for both planktonic growth and biofilm compared to control. Hence biofilm inhibition is higher than planktonic growth suggesting that silver nanoparticles are more resistant to biofilm forming cells. Instead, the difference was at least partially due to nanoparticle aggregation in biofilms because of the changes in ionic strength and interaction with various complexing agents such as extracellular polymeric substances released by the bacteria (Pugazhendhi et al., 2017). Nanoparticles at higher concentration may react with the microbial membrane and inactivate cell functions while small particles may enter the cells to disrupt microbial metabolism, making nanoparticles highly toxic (Shriniwas and Subhash, 2017). Therefore, the toxicity of silver nanoparticle may be controlled by particle size (cell internalization), silver release rate (particle stability), and surface characteristics such as surface film formation (Galocchio et al., 2017).

F. Ex-vivo Studies

The physical characteristics of incubated eggs are shown in Table 2. The hatch rate was increased for silver inoculated eggs up to 75% as compared to the control group (60%). High concentration of the silver nanoparticles (30 $\mu\text{g/kg}$) had increased hatch rate to 81% than the other two concentrations. It might be due to the increased immune activity against pathogenic microorganism. There was no difference in the growth performance and the hatch weight between the control and Ag inoculated eggs. This shows that silver nanoparticles were able to protect the chicken embryo from pathogenic infection which enables the healthy growth of offspring. Hatch rate was high in increasing concentrations of silver nanoparticles which means that the silver does not affect the growth and other biological activities of the embryo similar to previous studies (Galocchio et al., 2017; Pugazhendhi et al., 2017).

Parameters	Control	Trail 1 (10µg/kg)	Trail 2 (20µg/kg)	Trail 3 (30µg/kg)
Fertile eggs	30	30	30	30
Average egg weight (in gm)	45 ± 2.81	44 ± 5.60	45 ± 4.34	45 ± 1.64
Dead in germ	3	2	1	2
Fertility (%)	90	93.33	96.66	93.33
Hatchability on fertile eggs (%)	60	69	75	81
Chick weight (in gm)	28 ± 3.49	29 ± 5.61	29 ± 1.24	28 ± 9.57

Table 2: Results of silver nanoparticles inoculated egg hatchability

IV. CONCLUSION

Silver nanoparticles were synthesized using oxidation reduction method and characterized by UV Visible Spectroscopy, X-Ray Diffraction and SEM. The difference of silver nanoparticles toxicity between the biofilm and planktonic cells was evaluated, which shows that silver nanoparticles were more toxic to biofilm than planktonic cells. Silver nanoparticles are showing promising inhibitory effect against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Klebsiella pneumoniae* at much lower concentrations (< 10 µg). Further work can be carried out to evaluate the optimal lower concentrations of silver nanoparticles. Since these nanoparticles are causing lipid deformation in bacterial cells, their application in large scale should be investigated.

As the study suggests, the interface between nanotechnology and biology brings about a revolution in the field of livestock production. In poultry production, it is hypothesized that silver nanoparticles may affect intestinal microbial populations and improve the health and immunological status of the birds. This can provide the birds with an opportunity to expend less metabolic effort for immunological control purposes, and to use surplus nutrients for other physiological and productive purposes. In addition to that we proposed that the coating of anti-microbial paint containing silver nanoparticle in hatchery instrument prevents the microbial infections during hatching process and also silver nanoparticle incorporated glouse and mask will protect the poultry labours from the bacterial infection.

REFERENCES

- Carlson, C., Hussain, S.M., Schrand, M., Braydich-Stolle, L.K., Hess, K.L., Jones, R.L. and Schlager, J.J. (2008). Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species. *The Journal of Physical Chemistry B*, 112(43), 13608-13619.
- Das, R., Gang, S. and Nath, S.S. (2011). Preparation and antibacterial activity of silver nanoparticles. *Journal of Biomaterials and Nanobiotechnology*, 2, 472-475.
- Gallocchio, F., Biancotto, G., Cibin, V., Losasso, C., Belluco, S., Peters, R., van Bommel, G., Cascio, C., et al. (2017). Transfer Study of Silver Nanoparticles in Poultry Production. *Journal of Agricultural and Food Chemistry*, 65(18), 3767-3774.
- Grodzik, M. and Sawosz, E. (2006). The influence of silver nanoparticles on chicken embryo development and bursa of Fabricius morphology. *Journal of Animal and Feed Sciences*, 15, 111-114.

- Hajipour, M.J., Fromm, K.M., Ashkarran, A.A., de Aberasturi, D.J., de Larramendi, I.R., Rojo, T., Serpooshan, V., Parak, W.J. and Mahmoudi, M. (2012). Antibacterial properties of nanoparticles. *Trends in Biotechnology*, 1-13.
- Harrison, J.J., Rabiei, M., Turner, R.J., Badry, E.A., Sproule, K.M. and Ceri, H. (2006). Metal resistance in *Candida* biofilms. *FEMS Microbiology Ecology*, 55, 479 – 491.
- Jeevan, P., Ramya, K. and Rena, A.E. (2012). Extracellular biosynthesis of silver nanoparticles by culture supernatant of *Pseudomonas aeruginosa*. *Indian Journal of Biotechnology*, 11, 72-76.
- Karnib, M., Holail, H., Olama, Z., Kabbani, A. and Hines, M. (2013). The antibacterial activity of activated carbon, silver, silver impregnated activated carbon and silica sand nanoparticles against pathogenic *E. coli* BL21. *International Journal of Current Microbiology and Applied Sciences*, 2(4), 20-30.
- Kashid, S.B., Lakkakula, J.R., Chauhan, D.S., Srivastava, R. and Raut, R.W. (2017). Biocompatible antimicrobial cotton fibres for healthcare industries: A biogenic approach for synthesis of bio-organic-coated silver nanoparticles. *IET Nanobiotechnology*, 11(8), 1046-1051.
- Kim, J.S., Kuk, E., Yu, K.M., Kim, J.H., Park, S.J., Lee, H.J., Kim, S.H., Park, Y.K., et al. (2007). Antimicrobial effects of silver nanoparticles. *Nanomedicine: Nanotechnology Biology and Medicine*, 3, 95-101.
- Kota, S., Dumpala, P., Anantha, R.K., Verma, M.K. and Kandepu, S. (2017). Evaluation of therapeutic potential of the silver/silver chloride nanoparticles synthesized with the aqueous leaf extract of *Rumex acetosa*. *Scientific Reports*, 7(1), 11566.
- Lara, H.H., Trevino, E.N.G., Turrent, L.I. and Singh, D.K. (2011). Silver nanoparticles are broad-spectrum bactericidal and virucidal compounds. *Journal of Nanobiotechnology*, 9-30.
- Lara, H.H., Turrent, L.I., Trevino, E.N.G. and Padilla, C.R. (2010). PVP-coated silver nanoparticles block the transmission of cell-free and cell-associated HIV-1 in human cervical culture. *Journal of Nanobiotechnology*, 8-15.
- Percival, S.L., Bowler, P.G. and Russell, D. (2005). Bacterial resistance to silver in wound care. *Journal of Hospital Infection*, 60, 1-7.
- Prabhu, S. and Poulouse, E.K. (2012). Silver nanoparticles: Mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. *International Nano Letters*, 2, 32.
- Prabu, P.G., Selvisabhanayakam and Mathivanan, V. (2013). Antibacterial activity of silver nanoparticles against bacterial pathogens from gut of silkworm, *Bombyx mori* (L.) (Lepidoptera: Bombycidae). *International Journal of Research in Pure and Applied Microbiology*, 3(3), 89-93.
- Pugazhendhi, A., Prabakar, D., Jacob, J.M., Karuppusamy, I. and Saratale, R.G. (2017). Synthesis and characterization of silver nanoparticles using *Gelidium amansii* and its antimicrobial property against various pathogenic bacteria. *Microbial Pathogenesis*, S0882-4010(17)31356-6.
- Rai, M., Yadav, A. and Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnology Advances*, 27(1), 76-83.
- Reidy, B., Haase, A., Luch, A., Dawson, K.A. and Lynch, I. (2013). Mechanisms of silver nanoparticle release, transformation and toxicity: A critical review of current knowledge and recommendations for future studies and applications. *Materials*, 6, 2295-2350.
- Sawosz, E., Binek, M., Grodzik M, Zielinska, M., Sysa, P., Szmidi, M., Niemiec, T. and Chwalibog, A. (2007). Influence of hydrocolloidal silver nanoparticles on gastrointestinal microflora and morphology of enterocytes of quails. *Archives of Animal Nutrition*, 61, 444 – 451.
- Selvakumar, R., Aravindh, S., Ashok, A.M. and Balachandran, Y.L. (2013). A facile synthesis of silver nanoparticle with SERS and antimicrobial activity using *Bacillus subtilis* exopolysaccharides. *Journal of Experimental Nanoscience*, 9(10), 1-13.
- Shrinivas, P.P. and Subhash, T.K. (2017). Antioxidant, antibacterial and cytotoxic potential of silver nanoparticles synthesized using terpenes rich extract of *Lantana camara* L. leaves. *Biochemistry and Biophysics Report*, 10, 76-81.
- Soleimani, M. and Habibi, H.M. (2017). Biosynthesis of silver nanoparticles using *Chlorella vulgaris* and evaluation of the

Silver Nanoparticle: A Bactericidal Agent for Pathogenic Poultry Bacteria

antibacterial efficacy against *Staphylococcus aureus*. Avicenna Journal of Medical Biotechnology, 9(3), 120-125.

24. Sondi, I. and Sondi, B.S. (2004). Silver nanoparticles as antimicrobial agent: A case study on *E. coli* as a model for Gram-negative bacteria. Journal of Colloid and Interface Science, 275, 177–182.
25. Zhang, G., Niu, A., Peng, S., Jiang, M., Tu, Y., Li, M. and Wu, C. (2001). Formation of novel polymeric nanoparticles. Accounts of Chemical Research, 34, 249-256

