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Effect of Quercetin loaded Silver nanoparticles on Gram negative and Gram positive bacteria

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Microorganisms are increasingly becoming resistant to multiple antibiotics and search for effective antibacterial agents continue to challenge researchers in the respective domain. Silver nanoparticles (AgNPs) with physicochemical properties different from their bulk counterparts and show better cellular penetration resulting in higher antimicrobial activity. Here, we studied the antimicrobial activity of AgNPs in pure state as well as when coated with quercetin, and also the mechanism of action. Quercetin is known for its antimicrobial activity, hence applied here for coating it on AgNPs. Spherical AgNPs of size ranging between 10 and 28 nm were synthesised using chemical reduction method. The AgNPs show promising antimicrobial activity in Gram negative bacteria *Escherchia coli*; however, AgNPs loaded with quercetin did not enhance the antimicrobial activity of AgNPs. The mechanism of antimicrobial activity of AgNPs unravelled in this study was inhibition of bacterial catalase and decrease in membrane potential (MMP) of the bacterial cell with increase in concentration of AgNPs. Another mechanistic aspect was production of reactive oxygen species (ROS) by AgNPs resulting in apoptosis. However, there was no degradation of cellular DNA which suggests that it does not contribute to antibacterial activity of AgNPs.

Keywords: Catalase inhibition, DNA degradation, Mitochondrial membrane potential, Reactive oxygen species

Outbreak of infectious diseases caused by different pathogenic bacteria and the development of antibiotic resistance drive the the need for searching new antibacterial agents. In 2017, five years after the emergence of a strain of tuberculosis bacteria in India completely resistant to all available antimicrobial, the World Health Organization (WHO) published a list of 12 bacterial families, most of them Gram negative, which are resistant to carbapenems, colistin and third generation antimicrobial agent[1]. The WHO has also reported that if no action is taken on antimicrobial resistance, by 2030 it could force up to 24 million people into extreme poverty and drug-resistant diseases could cause 10 million deaths each year by 2050[2,3]. In the present scenario, nano scale materials have emerged up as novel tools for synthesizing antimicrobial agents owing to their high surface area to volume ratio and the unique chemical and physical properties.

The potential of silver as an antibacterial has been known since centuries and used to prevent bacteria from contaminating food, water and treating burns [4]. However, prolonged exposure to silver ions are known to cause adverse effects on human health causing respiratory, gastrointestinal, haematological issues among several other issues[5]. The properties of nanoparticles differ from both ion and bulk materials in terms of morphology and surface characteristics. They have distinct reactivity as well as catalytic activities, along with chemical and energetic properties[6,7]. A correlation between the shape of nanoparticles and their bactericidal efficiency is also reported[8,9]. Owing to their distinct antimicrobial properties, AgNPs can be loaded with other antimicrobial agents such as quercetin on surfaces to find application in drug delivery due to a large surface to volume ratio and efficient penetration into the cells[10].

The most common mode of action accepted is that AgNPs penetrate into the bacterial membranes causing collapse of the plasma membrane potential, interaction with DNA preventing normal replication and subsequent leakage of the intracellular materials which eventually leads to cell death[11-14]. They can also interact with the enzymes and induce generation of reactive oxidation species resulting in apoptosis[15-17], etc. The mechanism of antimicrobial action of AgNPs is still unclear and under investigation.

Flavonoids are used since long to heal certain diseases such as disturbances of the digestive system, inflammatory diseases, etc.[18]. A degree of synergy has been reported between the naturally occurring

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flavonoids and other antimicrobial agents against resistant strains of bacteria[19-22]. In the present study, AgNPs were synthesised by chemical reduction method and characterized using DLS, Zeta potential, SEM and FESEM. The antimicrobial activity of the AgNPs and quercetin loaded AgNPs were tested on Gram negative bacteria *Escherchia coli* and Gram positive bacteria *Staphylococcus aureus*. Also, we explored the mechanism of action by studying their interaction with DNA, influence on catalase kinetics, production of reactive oxygen species (ROS), and change in mitochondrial membrane potential (MMP).

Materials and Methods

Materials and bacterial strains

Chemicals such as silver nitrate and sodium borohydride were procured from Merck Life Sciences Pvt. Limited, Mumbai). Tryptone, Yeast Extract, Sodium chloride, Bacteriological agar, Tryptone soy agar, and Nutrient Broth without NaCl (pH 6.9) were obtained from Himedia Laboratories Pvt. Ltd., Mumbai. Quercetin, GenElute Bacterial Genomic DNA kit NA2100-1KT, 2′,7′-Dichlorofluorescin diacetate (DCFDA) and rhodamine 123 were obtained from Sigma-Aldrich Co., USA and H_2O_2 from Merck Specialities Pvt. Ltd., Mumbai.

Preparation of silver nanoparticles

$$Ag^{+} + BH_4^{-} + 3H_2O \rightarrow Ag^{0} + B(OH)_3 + 3.5H_2$$

AgNPs were synthesized using wet chemical reduction method with slight midifications[23]. A solution of 2 mM sodium borohydride and 1.0 mM silver nitrate were prepared and chilled to ensure a slow reaction. Silver nitrate solution was added drop wise to sodium borohydride solution till a stable straw coloured solution was obtained as observed in our earlier work[24]. Aliquots of 10 mL nanoparticles were centrifuged at 14000 rpm for 15 min at 4°C and their supernatant was discarded to concentrate the prepared nanoparticles. The nanoparticles obtained were further dried by keeping overnight in a desiccator and stored at 4°C.

Coating silver nanoparticles with flavonoid

A solution of 10^{-4} M quercetin was added to AgNPs in 1:1 ratio and incubated for 30-120 min in a shaker set at 200 rpm to allow loading of flavonoid on the nanoparticles[24].

Physical characterization of silver nanoparticles

UV-Vis absorption spectrophotometry:

UV-visible spectra were recorded with 1.0 cm path length for unloaded and loaded AgNPs, Cary 60 UV-Vis Spectrophotometer. AgNPs stored for up to 6 months in airtight containers at 4°C were also tested similarly. Milli Q water was used as a blank for all measurements[23].

Scanning electron microscopy (*SEM*)

SEM images were captured to analyze the size and surface morphology of the samples using a Zeiss Evo-MA 10 scanning electron microscope. The SEM samples were prepared by dropping 2 µL of test sample and spreading it into a thin film on thin cover slips cut into 5×5 mm pieces and drying in a desiccator overnight as used in our previous work[25].

Field emission scanning electron microscopy (*FESEM*)

FESEM images were captured to further elucidate the size and surface morphology of unloaded and loaded AgNPs using JEOL JSM-7600F FESEM machine. FESEM samples were prepared by dropping 2 µL of test sample and spreading it into a thin film on 5×5 mm pieces of thin aluminium sheet and drying in a desiccator overnight. The samples were thoroughly de-gassed prior to analysis to remove any residual moisture as followed in our previous work[25].

Disc diffusion test

Antibiotic impregnated discs are commonly used to test bacterial sensitivity to antimicrobial. The susceptibility of Gram negative bacteria *E. coli* (MCC 2412) and Gram positive bacteria *S. aureus* (MCC 2408) was examined on plates of nutrient agar without NaCl. AgNPs, both native and coated, were loaded on sterile discs and dried in a desiccator. From the stock 100 µL of (10^4-10^5 CFU/mL) bacteria was uniformly plated on each plate before supplementing them with discs infused with increasing amounts of unloaded AgNPs (10, 20, 30 and 40 µL) and a single disc with 40 µL of quercetin loaded AgNPs. The plates were then incubated at 37°C for 24 h; following which the inhibition zone surrounding the discs was measured[26] using a ruler with up to 1mm resolution and the measurements from three sets of each plate was averaged to obtain the zone of inhibition.

Determination of Minimum inhibitory concentration (MIC)

The lowest concentration of a substance that inhibits the growth of an organism is known as the minimum inhibitory concentration (MIC). Batch cultures containing varying amounts of unloaded AgNPs in four concentrations and a single concentration of loaded AgNPs each were used to obtain the logarithmic growth curve of *E. coli* and *S. aureus*. From an overnight bacterial culture the amount of inoculums required was determined such that the 0^{th} hour OD of the culture was approximately

0.5. Media corresponding to the calculated amount of inoculums to be used was discarded from each of the flasks prior to inoculation. Flask containing only inoculums but no AgNPs served as negative control. Test flasks containing inoculum were treated with 0.87, 1.54, 2.52 and 3.18 µg/mL AgNPs, and 1.54 µg/mL of quercetin loaded AgNPs, respectively, and their absorbance at 600 nm was recorded on an hourly basis[27]. The OD values of respective positive controls containing only test agents for each concentration but no inoculums were subtracted from the obtained OD of each test sample to obtain the true OD of each sample. All these experiments were performed in triplicates.

Gel electrophoresis of bacterial genomic DNA

E .coli was treated with AgNPs in increasing concentrations (1.54, 2.52 and 3.18 µg/mL). Damaging effect of AgNPs on bacterial DNA was studied by extracting bacterial genomic DNA from the silver nanoparticles treated bacterial culture (lane 2, 4 and 6) using slightly modified protocol described elsewhere[28] and Gen Elute Bacterial Genomic DNA kit and compared against genomic DNA extracted from untreated bacterial culture (lane 1, 3 and 5) on a 0.7% agarose gel electrophoresed at 80V for 24 h as described elsewhere[29]. The gel was visualized under UV light in a Gel Doc Bio-rad machine to check for bands.

Inhibition of catalase activity

E. coli cells (1.0 mL, 10^6 CFU/mL) in log phase were incubated with increasing concentrations of freshly prepared AgNPs for 15 min. *E. coli* cells from the same culture incubated without AgNPs served as control. Post incubation, the cultures were pelleted by centrifuging at 12000 rpm for 2 min. The supernatant of each tube was discarded and the pellet was re-suspended in 1.0 mL PBS of pH 7.4. The re-suspension was sonicated for 5 min using a probe sonicator to extract the cellular catalase.

The activity of catalase from untreated *E. coli* was followed using an absorption spectrophotometer as reported in our previous works[30]. H_2O_2 (0.15%) was allowed to react with 100 µL of cell extract in a quartz cell of path length 1.0 cm and the decrease in activity was recorded as absorbance at 10 s intervals at a wavelength of 240 nm at 25°C. The activity of catalase from *E. coli* treated with increasing concentrations of AgNPs was recorded in a similar manner. All these experiments were performed in triplicates.

Measurement of Reactive oxidative species (ROS)

Aliquots of *E. coli* cells (1.0 mL, 10^6 CFU/mL) in log phase were incubated with increasing concentrations of freshly prepared AgNPs for 15 min. *E. coli* cells from the same culture incubated without AgNPs served as control. After incubation, the cultures were pelleted by centrifuging at 12000 rpm for 2 min. The supernatant of each tube was discarded and the pellets were re-suspended in 800 µL PBS of pH 7.4. The resultant solution was incubated with the dye 2′-7′-dichlorodihydrofluorescein diacetate (DCFDA, 10 mM) at 37°C for 30 min[31]. The fluorescence emission intensity of the samples, when excited at 485 nm, was measured in a fluorescence-free quartz cell of 1.0 cm path length using a Perkin Elmer Fluorescence Spectrophotometer (LS-55) equipped with a 150 W Xenon flash lamp. Emission was recorded at 520 nm. The excitation and emission slits were set to 7 nm, the scan speed was set at 100 nm/min. The experiment was performed in triplicates.

Membrane potential assessment using Fluorimetry

Aliquots of *E. coli* cells (1.0 mL, 10^6 CFU/mL) in log phase were incubated with increasing concentrations of freshly prepared AgNPs for 15 min. *E. coli* cells from the same culture incubated without AgNPs served as control. Post incubation, the cultures were pelleted by centrifuging at 12000 rpm for 2 min. The supernatant of each tube was discarded and the pellets were re-suspended in 1.0 mL PBS of pH 7.4. The resultant solution was incubated with the dye rhodamine 123 (1.0 µL/mL) at 37°C for 30 min. The fluorescence intensity at 520 nm, when excited at 485 nm, was measured in a fluorescence-free quartz cell of 1.0 cm path length using a Perkin Elmer fluorescence spectrophotometer (LS-55) equipped with a 150 W Xenon flash lamp[32]. The excitation and emission slits were set to 7 nm, the scan speed was set at 100 nm/min. The experiment was performed in triplicates.

Results

Preparation of silver nanoparticles

Synthesis of AgNPs using the method described above yields a pale, straw yellow coloured solution with drop wise addition of 1.0 mM silver nitrate solution which immediately turns colourless on stirring. A stable, straw coloured solution that does not turn colourless with constant stirring is obtained after approximately 20 mL of 1.0 mM silver nitrate solution is added drop wise in 300 mL solution of 2 mM sodium borohydride. The appearance of the

stable, straw coloured solutions marks the end of the reaction.

Physical characterization of silver nanoparticles

UV-Vis absorption spectrophotometry

UV-Vis absorption spectrophotometry is one of the most widely used techniques for characterization of AgNPs. The absorption spectrum (Fig. 1) of the straw yellow coloured silver nanoparticles solution prepared by the chemical reduction method showed the characteristic surface plasmon absorption band with maxima at 398 nm. Absorption maxima at ~400 nm indicate the presence of spherical or roughly spherical AgNPs[24]. Quercetin loaded AgNPs demonstrated characteristic red shift in the spectra with absorption maxima at 294 nm.

Dynamic light scattering (*DLS*) *and Zeta potential*

The DLS size distribution of uncoated AgNPs synthesized using chemical reduction method is given in Fig. 2A. Nanoparticles of approximately 14 nm (±0.7) were most prevalent. Zeta potential analysis of synthesized AgNPs yielded a sharp peak at –27.3 mV, evidencing negative charge on the AgNPs in their dispersed state in the medium. The high negative surface potential exerts a high degree of repulsion between the particles and decreases aggregates. The DLS size distribution of AgNPs coated with quercetin in terms of number percent is shown in Fig. 2B. Coated nanoparticles of size 63.5 nm (±5.0) were most prevalent.

Scanning electron microscopy (*SEM*)

SEM images of quercetin loaded AgNPs (Fig. 3A) show that the loaded nanoparticles are spherical or roughly spherical in shape. Size of randomly chosen particles was measured (inset) and the sizes were found to be ~56 and ~67 nm.

Field emission scanning electron microscopy (*FESEM*)

FESEM images of unloaded AgNPs are shown in Fig. 3B. Surface morphology of the particles was found to be even and roughly spherical in shape. AgNPs of size ~22 and ~ 25 nm were most prevalent (inset). FESEM images of AgNPs loaded with quercetin are shown in Fig. 3C. The particles observed were found to be spherical or roughly spherical in shape. The sizes of some randomly chosen particles in the image are between 73 and 84 nm (inset).

Disc diffusion test

Disc diffusion test done on the LB plates of *E. coli* and *S. aureus* showed no zone of inhibition around AgNP loaded discs in any of the plates. In plates

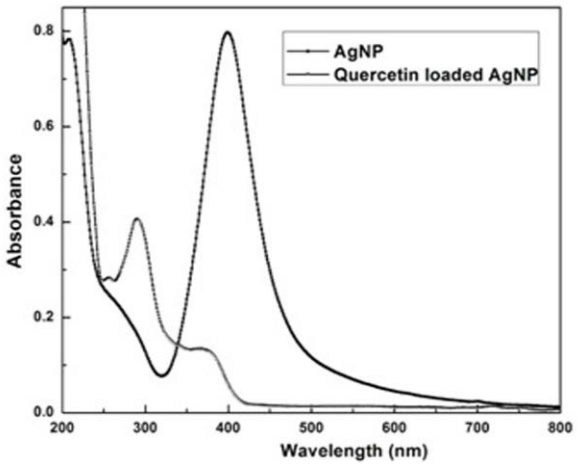

Fig. 1— UV-Vis absorption spectrum of freshly synthesized AgNPs.

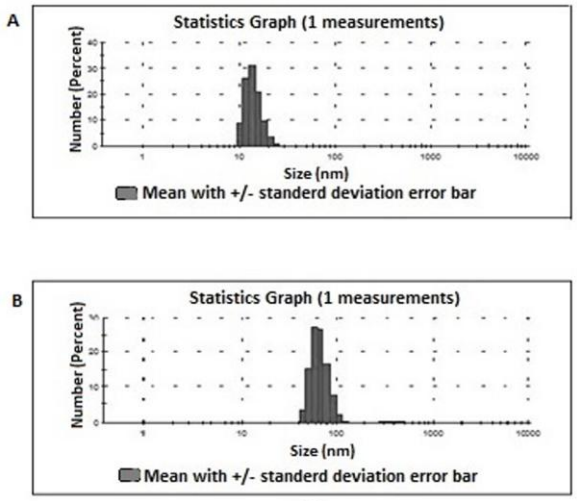

Fig. 2 — DLS Size distribution of (A) freshly prepared AgNPs; (B) quercetin coated AgNPs.

grown with *E. coli* containing nutrient agar without NaCl, a disc of diameter approximately 2 cm, known as the diameter of inhibition zone (DIZ), was observed around the disc containing 40 µL of re-suspended AgNPs as shown in Fig. 4A. There was no DIZ around discs containing lower amounts of AgNPs. No zone of inhibition was observed around plates containing discs loaded with quercetin loaded AgNPs Fig. 4B.

Determination of Minimum Inhibitory Concentration (MIC)

From batch culture studies on *E. coli* treated with increasing concentrations of AgNPs, the minimum inhibitory concentration calculated was approximately 1.54 µg/mL. Below this concentration no substantial difference in the growth profile of the cultures was observed. However, at the same concentration, no

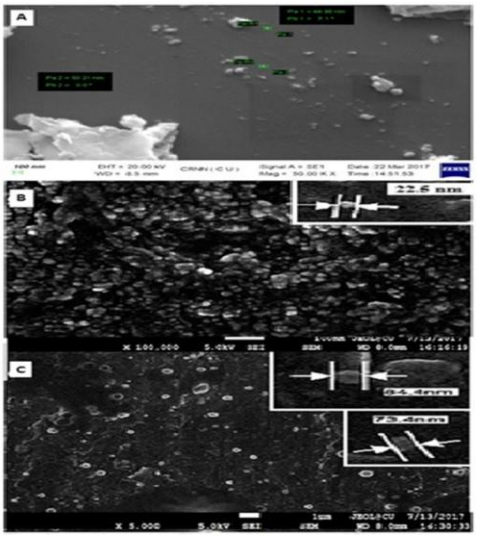

Fig. 3 — (A) Scanning electron microscopy (SEM) analysis of quercetin loaded AgNPs. Size of randomly chosen nanoparticles are approximately 45 nm and 50 nm (inset); (B) FESEM image of unloaded AgNPs of approximately 22 nm to 25 nm size (inset); and (C) FESEM image of loaded AgNPs of approximately 73 nm to 84 nm size (inset).

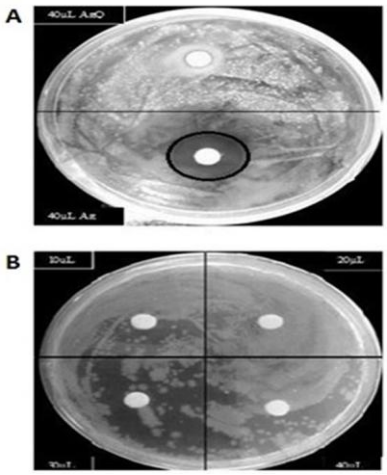

Fig. 4 — Disc diffusion test with (A) 40 µL AgNPs loaded with quercetin and 40 µL unloaded AgNPs tested on *E. coli*; and (B) 10, 20, 30 and 40 µL of uncoated AgNPs tested on *S. aureus*.

substantial difference was observed in the growth profile of *E. coli* treated with quercetin loaded AgNPs when compared to untreated *E. coli* cells (Fig. 5A). In batch culture studies conducted with *S. aureus* (Fig. 5B), no substantial difference was observed in the growth profile post treatment with AgNPs up to 3.18 µg/mL.

Gel electrophoresis of extracted DNA

Illustrated in Fig. 6, the bands of DNA from AgNPs (1.54, 2.52 and 3.18 µg/mL) treated *E. coli* (lanes 2, 4 and 6) were similar to the bands of DNA

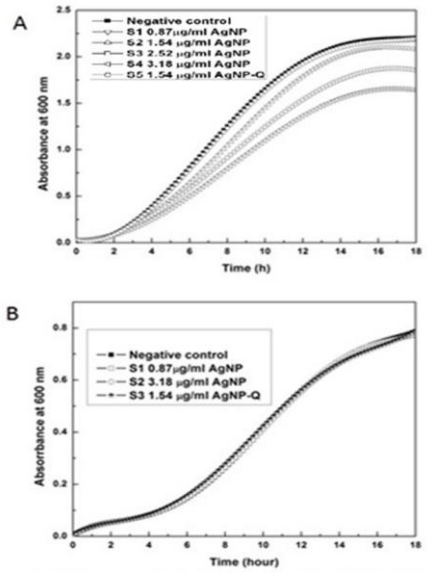

Fig. 5 — Representative batch growth profile of (A) *E. coli* cells; and (B) *S. aureus* cells treated with varying concentrations of unloaded and quercetin loaded AgNPs.

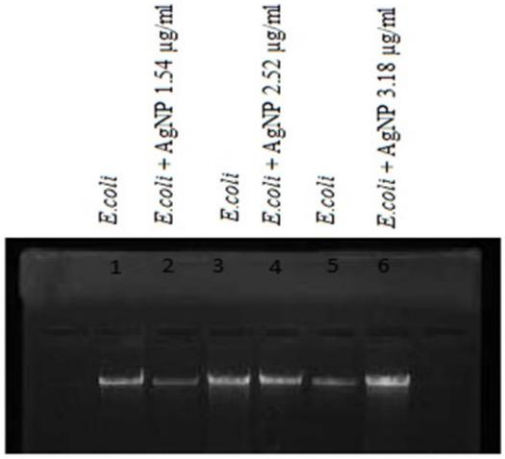

Fig. 6 — DNA gel electrophoresis of whole genomic DNA extracted from e. coli cells treated with 1.54, 2.52 and 3.18 µg/mL AgNPs compared against whole genomic DNA extracted from untreated cells.

from untreated *E. coli* cells (lanes 1, 3 and 5). The bands appear to be in the normal, open circular form.

Inhibition of catalase activity

Using absorption spectrophotometer, breakdown of H_2O_2 in presence of catalase was monitored at 240 nm. A progressive decrease in the rate of H_2O_2 degradation was observed upon addition of increasing concentrations of AgNPs as represented in Fig. 7A.

Measurement of Reactive oxidative species (ROS)

Intracellular concentration of ROS in *E. coli* cells treated with increasing concentrations of AgNPs was

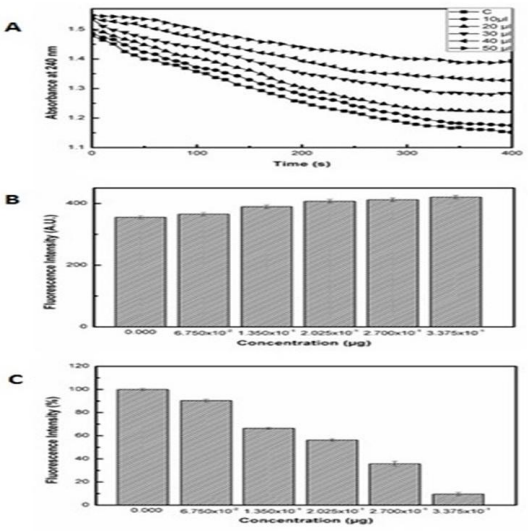

Fig. 7 — (A) Decay curves of H_2O_2 by catalase from *E. coli* cells treated with increasing amounts of silver nanoparticle solution compared against the activity of catalase from untreated cells; (B) Histogram representing intracellular ROS levels of *E. coli* cells treated with increasing concentration of AgNPs compared against untreated cells; and (C) Histogram representing changes in membrane potential of *E. coli* cells treated with increasing concentration of AgNPs compared against untreated cells.

compared to the intracellular levels of ROS produced by untreated *E. coli* cells in a fluorimetrically using DCFDA. Histogram (Fig. 7B) of the obtained results indicates that the concentration of intracellular ROS increases progressively with increasing concentration of AgNPs.

Membrane potential assessment using Fluorimetry

Spectrofluorimetric analysis of AgNPs treated *E. coli* cells compared to untreated *E. coli* cells showed a progressively reduced uptake of Rhodamine 123 with increasing concentrations of AgNPs. This suggests damage in the cell membrane on exposure to AgNPs. The results obtained are plotted as a histogram with the control intensity represented as 100% and the test intensities as relative percentages (Fig. 7C).

Discussion

Nano formulations of silver in the range of 1-100 nm is reported to have a wide range of applications such as antimicrobial, biosensor materials, composite fibres, cryogenic superconducting materials, cosmetic products, and electronic components[33]. Several studies have demonstrated that bactericidal properties of the AgNPs are strongly influenced by their shape, size, concentration, and colloidal state[34]. It has been found that reducing the size of AgNPs enhances their stability and biocompatibility[35]. Hence, it is necessary to design appropriate sized, shaped nanoparticles with desirable surface properties for use in a diverse range of clinical and therapeutic interventions. The present study was conducted in order to shed light on the underlying mechanism of antimicrobial action of free AgNPs and quercetin loaded AgNPs. Spherical shape of the unloaded and loaded AgNPs, prepared by the chemical reduction method, was revealed by SEM and FESEM and images shown in Fig. 3 (A-C), respectively. The absorption spectra of both unloaded AgNPs recorded absorbance maxima at 398 nm which indicates the presence of spherical or roughly spherical particles (Fig. 1). Red shift was observed when quercetin was coated on the AgNPs.

The antimicrobial actions of unloaded and loaded AgNPs with quercetin were tested by disc diffusion method on both gram negative *E. coli* (Fig. 4B) and gram positive *S. aureus* (Fig. 4B). In accordance with earlier studies[36], no zone of inhibition were observed with discs loaded with either of the AgNPs in Gram positive cells as they are more resistant to the nanotoxic effects due to thicker peptidoglycan layer. In nutshell, the lower response of Gram positive bacteria to antibiotic therapy can be explained on the basis of the fact that their cell wall is comparatively much thicker than that of Gram negative bacteria[37]. The thicker cell wall of Gram positive as well as the negatively charge of the peptidoglycan leave silver ions stuck onto the cell wall. *S. aureus*, a Gram-positive bacterium which possesses a thick cell wall and more peptidoglycan molecules prevent the action of the silver ions and renders bacterium comparatively more resistant to anti microbial therapy of the AgNPs[38].

In contrast, Gram negative bacteria are more susceptible to AgNPs-based antimicrobial therapy owing to less thicker cell wall and less peptidoglycan[39]. In addition, Gram negative bacteria contain lipopolysaccharides (LPS) in the cell membrane, which contributes to structural integrity of the membrane as well as protects the membrane from chemical attacks. The positive charge of LPS at physiological pH promotes adhesion of AgNPs and makes bacteria more susceptible to antimicrobial therapy. Several studies have shown the pronounced adhesion and deposition of AgNPs onto the cell surface, in particular, of the Gram negative bacteria due to the presence of LPS in their cell membrane[39]. These differences in structure, thickness and composition of cell can explain why Gram-positive *S. aureus* are less inhibited and Gram-negative *E. coli*

shows substantial inhibition even at low antibiotic concentrations[40]. Unloaded AgNPs were found to be toxic against *E. coli* with a minimum inhibitory concentration of 1.54 µg/mL. No significant antimicrobial effects of quercetin loaded AgNPs were observed. It is considered here that quercetin, a strong antioxidant, is reversing the bactericidal effect of AgNPs and not acting synergistically with it as anticipated by us. Also, its solubility poor in water and this is hindering its action. Gel electrophoresis bands of DNA extracted from untreated and AgNPs treated *E. coli* cells showed no variation (Fig. 6). As a result DNA degradation can be excluded as a probable mechanism of antimicrobial activity of AgNPs. Catalase inhibition was tested with AgNPs as shown in Fig. 7A to be effective. The function of catalase in cell is to regulate the oxidative species from causing deleterious damage to cells. When cellular catalase is inhibited its balance is disturbed and ROS production is recorded as shown in Fig.7B. ROS can damage intracellular structure mainly of mitochondria and add to apoptosis. The strong antibacterial, antifungal and antiviral activity of AgNPs is due to their ability to produce ROS, such as hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), hydroxyl radical (OH•), hypochlorous acid (HOCL) and singlet oxygen[41]. ROS when measured with the fluorescent probe DCFDA is observed to increase with increasing concentration of AgNPs as shown in Fig. 7B. This observation corroborates with reports in literature where, metallic nanoparticles were responsible for increase in ROS and increased toxic effect in the cells due to oxidative stress[42]. Apart from catalase, ROS in bacterial cells resulted from auto oxidation of NADH-dehydrogenase II in the respiratory chain[43]. Further, the antioxidant quercetin when loaded on the AgNPs reverses the effect of catalase inhibition by chelation[44-46]. Exposure to AgNPs also decreased the membrane potential in bacterial cells due to increased membrane permeability, as shown in Fig. 7C.These results suggest that the decrease in MMP is a parallel mechanism to catalase inhibition contributing to bacterial inactivity on exposure to AgNPs. This mechanism of action is similar to that of some already established antimicrobial agents which target cell membrane and alter bacterial membrane potential resulting in delocalization of membrane proteins due to change in membrane potential[41] and contributing to cellular damage.

Conclusion

The present study we explored the synergistic effects of AgNPs and quercetin as antimicrobial agents on Gram negative *E. coli* and Gram positive *S. aureus.* The underlying mechanism of AgNPs induced microbial activity was elucidated. Results have shown that AgNPs are effective on Gram negative *E. coli* and not on Gram positive *S. aureus.* AgNPs coated with quercetin did not enhance the efficacy of AgNPs antimicrobial activity in *E. coli* which is attributed to the antioxidant property of quercetin resulting in scavenging ROS and mitigating the bacterial damage. AgNPs antimicrobial activity is evidenced with inhibition of catalase, resulting in increased ROS population and consequent apoptotic cell death. Decrease in the membrane potential observed in *E. coli* leads to disruption of the membrane integrity and rendering bacterial cell death. No bacterial DNA damage was recorded and needs more focused experiments on DNA. Further studies are in progress to synthesise novel nano formulations to unravel the underlying mechanism of multidrug resistance in bacteria more effectively.

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Conflict of interest

Authors report no conflict of interest.

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