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INVESTIGATION OF ANTIMICROBIAL ACTIVITY OF SILVER NANO PARTICLE LOADED COTTON FABRICS WHICH MAY PROMOTE WOUND HEALING.

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ABSTRACT

Stable silver nanoparticles were prepared by the chemical process; these particles are incorporated in cotton fabrics. These cotton fabrics with silver nanoparticles are sterile and can be useful in to prevent or to minimize infection with pathogenic bacteria such as *Staphylococcus aureus* and *Escherichia coli*. The antimicrobial activity of Ag nanoparticles was investigated against *Escherichia coli* (MTCC-2940) Gram-negative bacterium, and *Staphylococcus aureus* (MTCC-74) Gram-positive bacterium. In these tests perform by the agar plates diffusion method were used and Ag nanoparticles loaded cotton fabrics to compare with Ampicillin loaded cotton. As results the growth-inhibitory effects on *S. aurous* were mild where as the growth-inhibitory effects on *E.coli* more potent and also perform dose dependent potency test of silver nanoparticles, when doses are increase so antimicrobial activity of Ag particles are increase. The bacteriostatic activity of Ag nanoparticles was evaluated by the help of SEM after 24 h incubation of bacterial strain inoculated cotton fabrics and calculated percent reduction of bacteria. Wound healing is a complex process and has been the subject of intense research for a long time. We therefore hypothesized that silver nanoparticles could improve the healing of burn wounds initially on the basis of the known antimicrobial property of silver.

KEYWORDS

Nanoparticles, Silver, Antimicrobial, Scaning Electron Microscopy, bacteriostatic activity.

INTRODUCTION

Most of the natural processes also take place in the nanometer scale regime. Therefore, a confluence of nanotechnology and biology can address several biomedical problems, and can revolutionize the field of health and medicine [1]. Nanotechnology is currently employed as a tool to explore the darkest avenues of medical sciences in several ways like imaging sensing [2], targeted drug delivery and gene delivery systems and artificial implants The new age drugs are nanoparticles of polymers, metals or ceramics, which can combat conditions like cancer and fight human pathogens like bacteria[4]. The development of new resistant strains of bacteria to current antibiotics has become a serious problem in public health; therefore, there is a strong incentive to develop new bactericides [3]. Bacteria have different membrane structures which allow a

general classification of them as Gram-negative or Gram positive. The structural differences lie in the organization of a key component of the membrane, peptidoglycan. Gram negative bacteria exhibit only a thin peptidoglycan layer (~2–3 nm) between the cytoplasmic membrane and the outer membrane; in contrast, Gram-positive bacteria lack the outer membrane but have a peptidoglycan layer of about 30 nm thick. Silver has long been known to exhibit a strong toxicity to a wide range of micro-organisms [5]; for this reason silver-based compounds have been used extensively in many bactericidal applications [6]. Silver compounds have also been used in the medical field to treat burns and a variety of infections. Several salts of silver and their derivatives are commercially employed as antimicrobial agents [7]. Commendable efforts have been made to explore this property using electron microscopy, which has revealed size dependent interaction of silver nanoparticles with bacteria. Nanoparticles of silver have thus been studied as a medium for antibiotic delivery and to synthesize composites for use as disinfecting filters and coating materials [8]. However, the bactericidal property of these nanoparticles depends on their stability in the growth medium, since this imparts greater retention time for bacterium and nanoparticle interaction. There lies a strong challenge in preparing nanoparticles of silver stable enough to significantly restrict bacterial growth.

The bactericidal effect of silver ions on micro-organisms is very well known; however, the bactericidal mechanism is only partially understood. It has been proposed that ionic silver strongly interacts with thiol groups of vital enzymes and inactivates them. Experimental evidence suggests that DNA loses its replication ability once the bacteria have been treated with silver ions. Our studies were carried out on both antibiotic resistant (ampicillin- resistant) and nonresistant strains of gram-negative (*Escherichia coli MTCC-74*) and a non-resistant strain of gram-positive bacteria (*Staphylococcus aureus MTCC-2940*). The effect of the nanoparticles was found to be significantly more pronounced on the gram-negative strains, irrespective of whether the strains were resistant or not, than on the gram-positive organisms. We attribute this enhanced antibacterial effect of the nanoparticles to their stability in the medium as a colloid, which modulates the phosphotyrosine profile of the bacterial proteins and arrests bacterial growth.

Materials and Methods

Chemicals Used

S.No	Name Of Chemical	Company Name
1	Silver nitrate	Thermo electron LLS India PVT. LTD. Navi Mumbai
2	Ammonia water	Glaxo Smith pharmaceutical LTD Mumbai
3	Formaldehyde	Central drug house (P) LTD. New Delhi
4	Polyvinyl pyrrolidone	Thermo electron LLS India PVT. LTD. Navi Mumbai
5	Acetone	Central drug house (P) LTD. New Delhi
6	Deionized water	

Instrument Used

S.No.	Instrument	Company Name
1	Autoclave	New India

2	B.O.D.	Delux Automatic B.O.D. Incudator
3	Laminar Flow	Narang Scientific works Pvt.Ltd New Delhi
4	Centrifugation Machine	REMI Centrifuge Mumbai India
5	Hot Oven	Narang Scientific works Pvt.Ltd New Delhi

Microbes stain used

S.No.	Stain Used	Stain No.
1	*Escherichia coli*	MTCC-74
2	*Staphylococcus aureus*	MTCC-2940

Synthesis of Silver Nanoparticle by Chemical Process [9]:To synthesize nano-sized Ag colloid, silver nitrate as a source of silver was dissolved in ammonia water. Formaldehyde as a reducing agent and polyvinyl pyrrolidone (PVP) as the stabilizing agent were used. The temperature of the reaction vessel was maintained at 40 ºC, and the pH of reaction solution was maintained at 10 ± 0.5. PVP coated silver colloids were washed with acetone. Finally, the silver gel was centrifugally separated and washed with deionized water and then dried at 150ºC for 3 hours.

Incorporation Of Silver Nanoparticle In Fabrics Cotton [10]: Cotton fabrics were washed, sterilized and dried before use Experiments were performed on samples with maximum dimensions of 5 cm × 5 cm. The final filtrate (100 ml, 240 ppm) obtained above was treated by centrifugation at 600 rpm for 24 h and dried at 70 °C. After dried perform the SEM of cotton.

Antimicrobial Activity of Silver Nitrate Nanoparticle Suspension on S.aureus inoculated agar plate (Diffusion Method)

- Taken Staphylococcus aureus stain no.MTCC-2940 inoculated plate.
- Cup cut in prepared medium using a sterile cork borer about 10 mm in diameter.
- The cut agar disc is removed by a vacuum device or a splayed-out pen nib.
- External diameter of 8 mm and height is about 10 mm.
- Then inject the plain sample in 1st and equal amount of ampicillin in 2nd and silver nanoparticle suspension 3rd cup.
- Then plate is keeping 24 hr in BOD for the incubation.
- After incubation inhibition of growth are observed as a clear zone of inhibition.
- Then measured the zone of inhibition.

Antimicrobial Activity of Silver Nanoparticle Suspension Loaded cotton fabrics on E.coli inoculated agar plate (Diffusion Method)

- Taken E.coli Stain No.MTCC-74 inoculated plate.
- Cup cut in prepared medium using a sterile cork borer about 10 mm in diameter.
- The cut agar disc is removed by a vacuum device or a splayed-out pen nib.
- External diameter of 8 mm and height is about 10 mm.
- Then inject the plain cotton in 1st and equal amount of ampicillin incorporated cotton in 2nd and silver nanoparticle suspension incorporated cotton in 3rd cup.
- Then plates are keeping 24 hr in BOD for the incubation.
- After incubation inhibition of growth are observed as a clear zone of inhibition.
- Then measured the zone of inhibition.

Dose dependent antimicrobial activity of silver nanoparticle on E.coli Stain No.MTCC-74 inoculated agar plate

- Taken E.coli (MTCC-74) stain inoculated three plates.
- 1st plate taken untreated of silver nanoparticle.

- 2nd plate taken 5 ppm suspension of silver nanoparticle treated.
- 3rd plate taken 10 ppm suspension of silver nanoparticle treated.
- Then inspected to the growth of the E.coli in the 1st, 2nd, and 3rd, plate.

Antimicrobial Activity Of Silver Nitrate Nanoparticle Suspension Loaded cotton fabrics by SEM analysis method [10]

The antibacterial behavior of the fabrics were evaluated against *Staphylococcus aureus* (MTCC 2940), a Gram-positive bacterium. The cotton fabrics were inoculated on agar plates inoculated with *S. aureus*. The inoculums were 1.3–1.6 105/ml. After 24 h, the plates were sterilized and the cotton fabrics were analyzed by Scanning Electron Microscopy (SEM).

In order to study the antimicrobial activity of the fabrics, squares of 1 cm of each fabric were prepared in aseptic manner. Each square was placed in a sterile vial and the fabrics subjected to pretreatment with 800 *u*l distilled water for 10 min tryptone soy broth (2.2 ml) was then added to each vial to make up to a total volume of 3 ml. An aliquot (10 *u*l) of *S. aureus* suspension was added to each vial (1.6×105/ml) containing the fabrics. Control broths with and without bacterial inoculation were also included. The vials were then incubated with agitation at 35 ^{O}C, 220 rpm. Aliquots of 10 *u*l broth were sampled at 24 h and serial dilution for the aliquots was prepared in broth. Duplicate aliquots (50 *u*l) of the serially diluted samples were spread on to plates. The plates were incubated at 35 ^{O}C and bacterial counts were performed. The bacteriostatic activity was evaluated after 24 h and calculated percent reduction of bacteria. Using the following equation: $R(\%)=[A-B]/A]\times100$.Where R=the reduction rate, A = the number of bacterial colonies from untreated fabrics, and B = the numbers of bacterial colonies from treated fabrics.

RESULT

Prepared Silver nano-particles: Silver nanoparticle was successfully produced less than 10nm in size (Fig.1) determined by the size of nano-particle help of SEM analysis.

Effect of Incorporation Of Silver Nanoparticle In Fabrics Cotton: Nano-particles are successfully incorporated in the cotton fabrics by the help of centrifugation detected by SEM analysis (Fig.1). And size of particles is funded to be 5 nm.

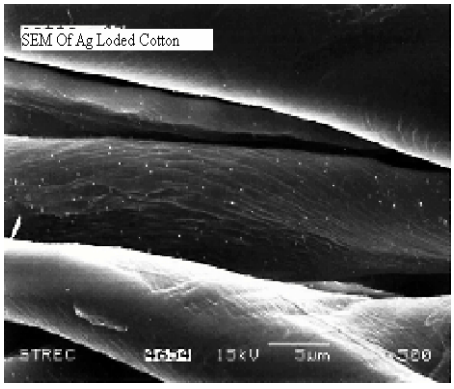

Fig. 1. ***SEM micrograph of silver nanoparticles loaded cotton fabric***

Effect on antimicrobial Activity of Silver Nanoparticle Suspension on *S.aureus* inoculated agar plate (Diffusion Method):After the 24 hr incubation of *S.aureus* inoculated plate we observed the zone of inhibition of the silver-nanoparticle suspension and ampicillin. Then measured the zone of inhibition.

Table 1.
Zone of inhibition of silver nano-particle suspension and ampicillin

S.No	Concentration of antibiotic	Name of microbes	Diameter of inhibition
1	Plain sample	staphylococcus aureus (MTCC-2940)	No inhibition
2	Ag nanoparticles suspension *50ug*/ml	staphylococcus aureus (MTCC-2940)	12mm
3	Ampicillin *20ug*/ml	staphylococcus aureus (MTCC-2940)	15mm

We had successfully measured the inhibition of growth of silver-nanoparticle suspension (table no.1). And clear observe the zone of inhibition (Fig No. 2).

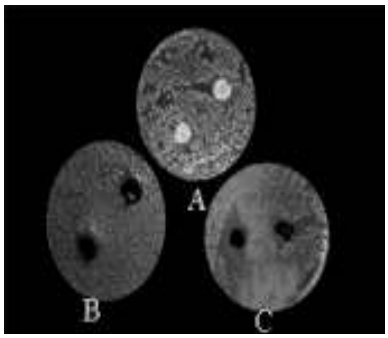

Fig.No.2*: zone of inhibition on S.aureus inoculated agar plate*
(A) Plain sample (B) Ag nanoparticles suspension 50ug/ml (C) Ampicillin 20ug/ml

Effect on Antimicrobial Activity of Silver Nanoparticle Suspension Loaded cotton fabrics on *E.coli* inoculated agar plate (Diffusion Method):
After the 24 hr incubation of *E.coli* inoculated agar plate we observed the zone of inhibition of the silver-nanoparticle suspension and ampicillin incorporated fabrics cotton. Then measured the zone of inhibition.

Table 3.
zone of inhibition of silver nano-particle incorporated cotton

S.No	Concentration of antibiotic	Name of microbes	Diameter of inhibition
1	Plain sample	E.coli Stain No.MTCC-74	No inhibition
2	Ag nanoparticles incorporated cotton	E.coli Stain No.MTCC-74	11mm
3	Ampicillin incarporated cotton	E.coli Stain No.MTCC-74	12mm

We had successfully measured the inhibition of growth of silver-nanoparticle incorporated cotton fabrics and ampicillin incorporated cotton fabrics (table no.2). And clear observe the zone of inhibition (Fig No. 3).

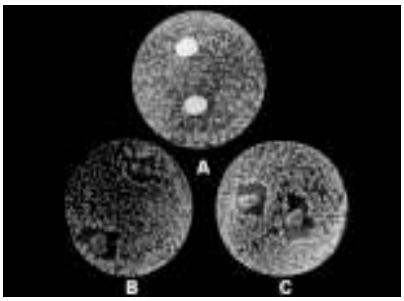

Fig 3. ***Agar diffusion test for E.coli inoculated agar plate (Stain No.MTCC-74)***
(a) Plain cotton (b) silver nano particle incorporated cotton (c) ampicillin incorporated cotton

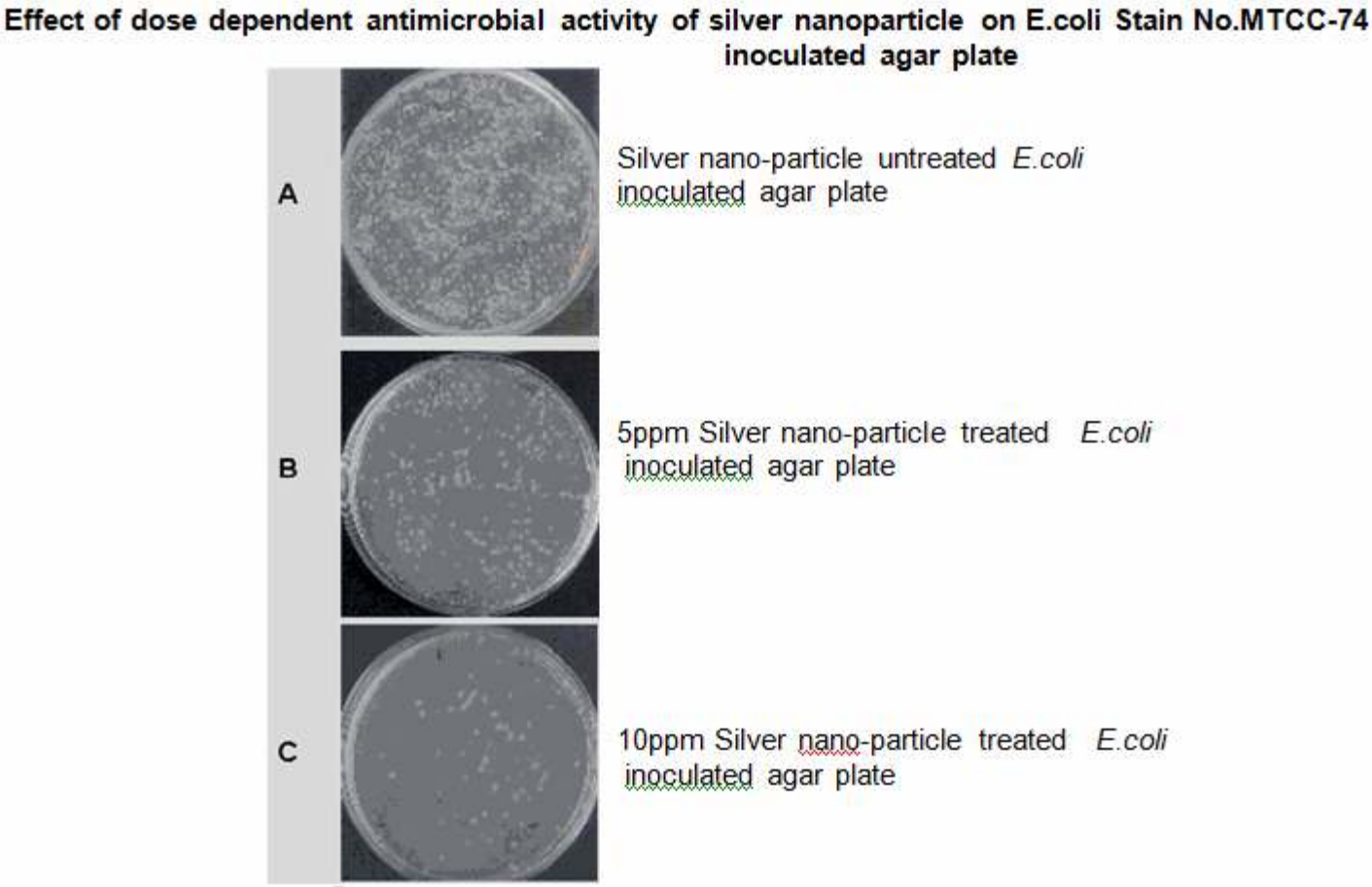

Fig 4. ***Dose dependent antimicrobial activity of Silver nano-particle***

We had found when the dose of silver nano-particle are increase in culture medium so the growth of *E.coli gram*-negative bacterium (MTCC-74) are reduced

Effect of antimicrobial Activity Of Silver Nitrate Nanoparticle Suspension Loaded cotton fabrics by SEM analysis method: The antibacterial behavior of the fabrics were evaluated against *Staphylococcus aureus* (MTCC 2940), a Gram-positive bacterium. We had found to be % reduction of silver nano-particle by the SEM.

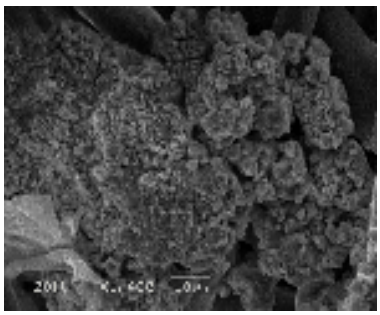

Fig 5. *S.aureus inoculated silver nano-particle untreated fabric cotton SEM*

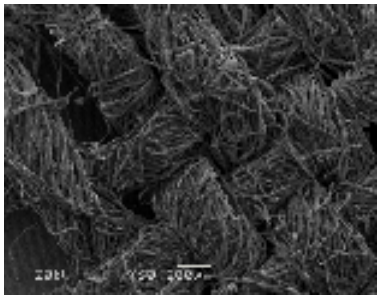

Fig 6. *S.aureus inoculated silver nano-particle incorporated fabric cotton SEM*

Calculation for percentage reduction of silver nano-particle treated fabrics cotton

- *Bacterial colonies from untreated fabrics found to (A)=173 per cm*
- *Bacterial colonies from Ag treated fabrics found to (B)=19 per cm*

R(%)=[173-19]/[173] X *100*
R(%)=89%
%Reduction are found to be 89%

- For the SEM analysis of *Staphylococcus aureus* (MTCC 2940), a Gram-positive bacterium inoculated cotton in which silver nano-particle is incorporated and calculated the percentage reduction.

DISCUSSION

From this study we concluded that the in situ formation of Ag nanoparticles in a chemical process and incorporated in the fabrics cotton. The network of cotton fabric was an effective method for the preparation of antibacterial fabrics. The almost uniform distribution of narrow dispersed Ag nanoparticles is a major advantage of this method. These fabrics show biocidal action against the bacteria *E. coli* and S. *aureus*, thus showing great potential to be used as an antiseptic dressing or bandage, which is in high demand for biomedical applications.

Agar culture medium had a pale yellow color [Fig No.2] before the addition of Ag+ ions which changed to a brownish color on completion of the reaction with Ag+ ions for 28 h. The appearance of a brownish color in solution containing the biomass is a clear indication of the

formation of silver nanoparticles in the reaction mixture [15].

The silver nanoparticles synthesized and analyzed in this report were found to have stronger antibacterial potency than those described in the earlier reports [11, 12]. The effect was dose dependent and was more pronounced against gram-negative organisms than gram-positive ones. The antibacterial effect of nanoparticles was independent of acquisition of resistance by the bacteria against antibiotics. The major mechanism through which silver nanoparticles manifested antibacterial properties was by anchoring to and penetrating the bacterial cell wall, and modulating cellular signalling by dephosphorylating putative key peptide substrates on tyrosine residues. However, further studies must be conducted to verify if the bacteria develop resistance towards the nanoparticles and to examine cytotoxicity [14] of nanoparticles towards human cells before proposing their therapeutic use.

Silver nanoparticles exhibit a broad size distribution and morphologies with highly reactive facets. The major mechanism through which silver nanoparticles manifested antibacterial properties is by anchoring to and penetrating the bacterial cell wall, and modulating cellular signalling by dephosphorylating putative key peptide substrates on tyrosine residues. Silver nanoparticles act primarily in three ways against Gram-negative bacteria:

(1) Nanoparticles mainly in the range of 1–10 nm attach to the surface of the cell membrane and drastically disturb its proper function, like permeability and respiration;

(2) They are able to penetrate inside the bacteria and cause further damage by possibly interacting with sulfur- and phosphorus-containing compounds such as DNA;

(3) Nanoparticles release silver ions, which have an additional contribution to the bactericidal effect of the silver nanoparticles. Although bacterial cell lyses' could be one of the reasons for the observed antibacterial property, nanoparticles also modulate the phosphotyrosine profile of putative bacterial peptides, which could thus affect bacterial signal transduction and inhibit the growth of the organisms.

The effect is dose dependent and is more pronounced against gram negative organisms than gram-positive ones. The antibacterial effect of nanoparticles is independent of acquisition of resistance by the bacteria against antibiotics. However, further studies must be conducted to verify if the bacteria develop resistance towards the nanoparticles and to examine cytotoxicity [15] of nanoparticles towards human cells before proposing their therapeutic use. The reactive metal oxide nanoparticles show excellent bactericidal effects, it is of great interest to investigate the use of other inorganic nanoparticles as antibacterial materials. Much less is known about the biocidal effects of noble metal particles. It has been known for a long time that silver ions and silver compounds are highly toxic to most bacteria, while just a few rare strains are silver-resistant. Recently it was shown that highly concentrated and nonhazardous nanosized silver particles can easily be prepared in a cost-effective manner and tested as a new type of bactericidal nonmaterial. In this study, the application of silver nanoparticles as an antimicrobial agent was investigated by growing *E. coli* on agar plates supplemented with silver nanoparticles. When nanoparticles were present on agar plates, they could completely inhibit bacterial growth. However, inhibition depends on the concentration of the silver nanoparticles (Fig No.6). When the dose of nano-particles are increase so the growth of microbes are reduce.

SEM microscopy shows macroscopic aggregates composed of nanosized silver particles and dead bacterial cells (fig No.6). Obviously, these particles have only a limited use as biocidal materials in liquid systems because of their low colloidal stability. 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. In addition, it was also shown that Ag+ binds to functional groups of proteins, resulting in protein denaturation. The obvious question is how nanosize silver particles act as biocidal material

against *E. coli*. There are reports in the literature that show that electrostatic attraction between negatively charged bacterial cells and positively charged nanoparticles is crucial for the activity of nanoparticles as bactericidal materials However, silver particles used in this study are negatively charged. While the mechanism of the interaction between these particles and the constituents of the outer membrane of *E. coli* is unfortunately still unresolved, it would appear that, despite their negative surface charge, they somehow interact with "building elements" of the bacterial membrane, causing structural changes and degradation and finally, cell death. Indeed it is clear that treated bacteria also show significant changes in and damage to membranes, which are recognized by the formation of "pits" on their surfaces. A similar effect was found to be when *E. coli* bacteria were treated with highly reactive metal oxide nanoparticles. 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. It is well known that the outer membrane of *E. coli* cells is predominantly constructed from tightly packed lipopolysaccharide (LPS) molecules, which provide an effective permeability barrier.

CONCLUSION

This study demonstrated the possibility of use biological synthesized silver nanoparticles and their incorporation in fabrics cotton, providing them sterile properties. The cotton fabrics incorporated with these silver nanoparticles exhibited antibacterial activity against *S. aureus and E.coli.* Silver nano-particles are more potent for the gram-negative bacterium.Wound healing is a complex process and has been the subject of intense research for a long time. We therefore hypothesized that silver nanoparticles could improve the healing of burn wounds initially on the basis of the known antimicrobial property of silver. Nonetheless, our findings reported herein not only confirm the efficient antimicrobial property of silver nanoparticles, but also implicate the ability of silver to modulate the cytokines involved in wound healing.These results have given insight into the actions of silver and have provided a novel therapeutic direction for wound treatment in clinical practice.

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