Global Veterinaria 12 (6): 745-749, 2014
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2014.12.06.83198

Potential Antibacterial Properties of Silver Nanoparticles Conjugated with Cow and Camel Milks

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Abstract: Biosynthesized silver nanoparticles (AgNPs) were synthesized with the aid of a novel, non-toxic, eco-friendly biological material. In this study, the potential antimicrobial activity of aqueous nanoparticles conjugated with cow milk and camel milk were investigated against six bacterial strains of Gram-positive and Gram-negative bacteria. The Gram-positive bacteria were represented by *Staphylococcus aureus* (ATCC 25923) and *Streptococcus pyogenes* while the Gram-negative bacteria were represented by *Klebsiella pneumonia (*ATCC 27736*), Pseudomonas aeruginosa* (ATCC 27853) *Salmonella typhi* (ATCC 19430) and *Escherichia coli* (ATCC 35218). The results indicated that aqueous silver nanoparticles conjugated with cow and camel milks were effective antibacterial agents against the tested pathogenic bacterial strains. But the aqueous silver nanoparticles conjugated with camel milk are more effective than its counterpart with cow milk. The results indicated that aqueous silver nanoparticles conjugated with cow and camel milks can be used as complementary antimicrobial agents against pathogenic bacterial strains.

Key words: Antibacterial Properties · Nanoparticles · Cow Milk · Camel Milk

INTRODUCTION

Silver nanoparticles are heterogeneous group of substances with various sizes (1-100 nm) and structures. Among metallic nanoparticles, silver is most widely recognized for its application in medical and other scientific fields including clinical diagnostics and therapy [1-11].

The biological synthesis of AgNPs has received considerable attention and offers many advantages. Various techniques, including chemical and physical processes, are developed to prepare silver nanoparticles [12]. The milk, as an economic and easily available product was used for the synthesis of AgNPs. The proteins present in cow milk could be responsible for Ag+ ions reduction [13].

The control of bacterial diseases is getting more difficult due to the growing resistance to antibiotics [14, 15]. Meanwhile, the use of synthetic drugs has severe side effects especially with those tested for the dangerous diseases. Therefore, it is useful to return to the natural products and medicinal plants to overcome the side effects of chemicals. The current control measures are concentrated mostly on preventing dissemination of disease to uninfected animals and humans by using the natural products [16-19]. The application of nanoparticles focused on the control of diseases. Silver nanoparticles, in particular, exhibited different activities including antifungal [20] antibacterial [21-25] and antiviral activities [26]. Thus, the objective of the present study was to biosynthesis AgNPs using cow milk and camel milk to evaluate the antibacterial activity of the synthesized AgNPs *in vitro*.

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MATERIALS AND METHODS

Synthesis of Silver Nanoparticles: Silver nitrate was purchased from Sigma-Aldrich (St. Louis, MO). Cow milk and camel milk were procured from local market. Each of cow milk and camel milk (4 mL) was mixed with 96 mL of 1 mM silver nitrate solution and the resulting mixture was incubated for 8 h in rotatory shaker (180 rpm) at room temperature according to the method described by Lee *et al.* [13] Reduction of silver ions in the reaction mixture was monitored by change in color of the reaction mixture from milky white to dark brown. The reaction product was separated by centrifugation at 13000 rpm for 15 min and purified by dispersion of the pellet in autoclaved water. The processes of centrifugation and re-dispersion were repeated several times to ensure better separation of the free entities from the AgNPs. The obtained nanomaterial was freeze dried at -80 °C and used for characterization studies. Autoclaved water was used as a control negative for this experiment.

Characterizations of the Biologically Synthesized Silver Nanoparticles: UV-VIS spectra were performed with a UV-Visible spectrophotometer Model V-570 UV/VIS/NIR, operating in the absorption mode in the range of 200-1000nm. FTIR spectrum was obtained using a Fourier-transform infrared instrument in the spectral range of 4000-400 cm^{-1} at room temperature. The surface morphologies and sizes of the AgNPs were examined using biological transmission electron microscopy (JEOL JEM 2010 transmission electron microscope) and X-ray diffractometer as Moharram *et al.* [23].

Bacterial Strains: Six bacterial species including Gram-positive and Gram negative were used. These bacteria were kindly provided from Department of Zoonotic Diseases, National Research Center, Egypt and Department of Botany, Faculty of Sciences, Al Azhar University, Assuit Branch, Egypt. The Gram-positive bacteria were *Staphylococcus aureus* (ATCC 25923) *Streptococcus pyogenes* (human isolate), while the Gram-negative bacteria were *Klebsiella pneumonia (*ATCC 27736*), Pseudomonas aeruginosa* (ATCC 27853) *Salmonella typhi (ATCC 19430)* and *Escherichia coli* (ATCC 35218).

Antibacterial Assay: The pathogenic bacterial strains were used to determine the antibacterial activity of the silver nanoparticles conjugated with cow milk and camel milk. The bacterial cultures were maintained on nutrient agar (NA) that contained peptone, 5.0; meat extract, 1.0; yeast extract, 2.0; sodium chloride, 5.0; and agar, 15.0 g per liter of distilled water [27]. The bacterial suspension was prepared and adjusted by comparison against 0.5 McFarland turbidity standard ($1.5x10^8$ CFU/mL) tubes. It was further diluted to obtain a final count of $5X10^6$ CFU/mL. The tested bacterial suspensions (100 µl) containing 10^6 CFU/mL was spread on NA plates. A total volume of (50 µl) of freshly prepared silver nanoparticles (synthesized with 10 mL of aqueous silver nanoparticles conjugated with cow milk and camel milk) were added onto a filter paper with diameter of 5.5 mm. The filter paper was then put into the seeded plates. Control milk samples free of the silver nitrate were used to assess the antimicrobial activity of the aqueous silver nanoparticles conjugated with cow milk and camel milk. The samples were initially incubated for 15 min at 4ºC (to allow diffusion) and then at 37°C for 24 h to allow the growth of the bacterial cultures. Positive test results were scored when a zone of inhibition was observed around the filter paper after the incubation period. The antibacterial activity was evaluated according to the criteria: zone of inhibition range >18 mm showed significant activity, 16-18 mm good activity, 13-15 mm low activity, 9-12mm non-significant activity and <8 mm no activity [28]. The cultures that were inhibited by the aqueous silver nanoparticles conjugated with cow milk and camel milk were also subjected to the antimicrobial activity experiment with gentamicin as standard antibacterial drug [29].

Statistical Analysis: The results obtained in the present study were represented as means ± standard error and were analyzed using analysis of variance (ANOVA). The significancet difference between means at $P<0.05$ was calculated using the Duncan Multiple Range Test [30].

RESULTS

Scanning electron microscopy dispersive spectroscopy peak, approximately at 3ke V, confirmed the presence of silver nanoparticles. Transmission electron microscopy showed that nanoparticles are mostly circular with an average size of 30-90 nm.

Table 1 shows the inhibition zone of bacterial growth caused by silver nanoparticles conjugated with cow or camel milk, as compared with those caused by Gentamicin. Sliver nanoparticles conjugated with cow milk was the most effective against *S. aureus, S. pyogenes* and *K. pneumonia* with zones of inhibition of 46.7 ± 0.1 mm, 51.4 ± 2.9 mm and 39.5 ± 0.9 mm respectively. Nanoparticles conjugated with camel milk followed that

Table 1: Zone inhibition of different bacteria by the aqueous nanoparticles silver conjugated with cow milk and camel milk

		Silver nanoparticles conjugated	
Pathogens tested	Gentamicin (400 units/mL)	Cow milk	Camel milk
Staphylococcus aureus	32.5 ± 0.9	46.7 ± 0.1	40.1± 0.4
Streptococcus pyogenes	30.6 ± 0.1	51.4 ± 2.9	40.5 ± 0.7
Klebsiella pneumonia	22.5 ± 0.1	39.5 ± 0.9	10.6 ± 0.0
Pseudomonas aeruginosa	26.5 ± 0.3	51.7± 0.6	55.7± 0.5
Salmonella typhimurium	46.7 ± 0.3	17.5 ± 0.5	24.9 ± 0.1
Escherichia coli	12.5 ± 0.7	35.2± 0.2	45.7± 0.2

Growth Inhibition = Inhibition of the growth measured by mm.

conjugated with cow milk in its activity against *S. aureus* and *S. pyogenes* with inhibition zones of 40.1 ± 0.4 mm and 40.5 ± 0.7 mm respectively. While Gentamicin was more effective against *K. pneumonia* than silver nanoparticles conjugated with camel milk but still less effective than that conjugated with cow milk with inhibition zone of 22.5 ± 0.1 mm. Meanwhile, *P. aeruginosa* and *E. coli* showed the most sensitivity to silver nanoparticles conjugated with camel milk with inhibition zones of 55.7 ± 0.5 mm and 45.7 ± 0.2 mm respectively. Gentamicin showed the highest activity against *S. typhi* with inhibition zone of 46.7 ± 0.3 mm followed by silver nanoparticles conjugated with camel milk with inhibition zone of 24.9±0.1 mm.

DISCUSSION

The silver nanoparticles conjugated with cow and camel milks displayed antimicrobial activity towards the tested pathogenic strains including *S. aureus, S. pyogenes, K. pneumonia, P. aeruginosa, S. typhi* and *E. coli*. In similar studies the antibacterial activity of silver nanoparticles conjugated with chitosan and aloe leaf extract against Gram-positive and Gram-negative bacteria including *S. aureus* and *E. coli* was documented [23, 24]. Combination of silver nanoparticles with lysozyme enzyme, a well known antimicrobial enzyme, did not affect the hydrolase function of the enzyme and its antimicrobial activity was retained against *E. coli*, *S. aureus*, *Bacillus anthracis* and *Candida albicans* [31].

The potential antimicrobial activities displayed by silver nanoparticles conjugated with cow and camel milk have made them promising candidates as new generation antimicrobials [32,33]. The ability of aqueous silver nanoparticles conjugated with cow and camel milk to inhibit the growth of the bacterial strains tested in this experiment may be due to the combined effect of both sliver nanoparticles and milk. Silver in its bulk form exhibits antibacterial activity. Such activity may be due to the physical structure of silver nanoparticles in part and due to the release of antibacterial ions from the nanoparticles surfaces as reviewed by Seil and Webster [11]. The surface of nanoparticle could easily form a layer of water, thus silver ions could be released into the water. The main composition of bacteria cell wall is phospholipid bilayers and protein molecules and the phosphate in phospholipid molecules possess negative electricity, leading the whole cell membrane to be negatively charged. Therefore, the silver ions with positive electricity are able to bind to bacteria cell wall quickly, which leads the structures of bacteria cell wall to be changed and damaged. Moreover, Ag ions interact with sulfhydryl (-SH) group of bacterial proteins and DNA bases. This interaction leads to the inhibition of respiratory processes [34] or DNA unwinding [35]. Inhibition of cell division and damage to *P. aerugenosa* spheroplasts envelopes with silver was documented earlier [36] and interaction with hydrogen bonding processes has been demonstrated to occur [37].

On the other hand, cow milk and camel milk contain proteins with their amphoteric effect that help in penetration through bacterial membrane. The milk of mammals is armed with natural inhibitory systems against microbial contamination including the lacto peroxidase/thiocyanate/hydrogen peroxide system (LPS), lactoferrins, lysozyme, immunoglobulins and free fatty acids [38,39]. Adlerova *et al.* [40] and González-Chávez *et al.* [41] stated that lactoferrin possesses various biological functions, including roles in iron metabolism, cell proliferation and differentiation, antibacterial, antiviral and anti-parasitic activity. Many of these functions do not appear to be connected with its iron binding ability.

The results indicated that silver nanoparticles conjugated with cow and camel milk could beuseful complementary antimicrobial agents against pathogenic bacteria. Pal *et al.* [42] demonstrated that there is shape-dependence of the antibacterial activity of silver nanoparticles against *E. coli* as a representative of Gram-negative bacteria. So, further studies are needed to evaluate the effect of different parameters including the shape and size of the nanoparticles on their antimicrobial activity upon conjugation with cow and camel milk.

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