Antimicrobial Activity and Cytotoxicity of Silver Nanoparticles Formulated Cream Against *Staphylococcus aureus* Dermal Infection in Albino Rats

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Spherical shaped 10–20 nm average size silver nanoparticles were prepared by chemical precipitation method and tested *in vitro* as antimicrobial agent against *Staphylococcus aureus*. The particles were characterized via UV-Vis spectrophotometer and TEM photography. 2% (w/v) silver nanoparticles cream was formulated to be used as topical treatment of *Staphylococcus aureus* infected wound in albino rats. The commercially available antibiotic cream fucidin was used as a standard antibiotic. The infection and treatment stages were followed by morphology, histopathology and immunological analysis. The prepared cream showed high healing effect within 4–6 days with no noticeable morphological change in the skin. In addition silver nanoparticles affect the humoral immunity by increase the serum level of IgG, IgA, IgM as comparing with infected-non treated group. The histopathological examination showed formation of inflammatory cells in infected wounds then significant decrease of the inflammation after treatment with silver nanoparticles cream.

**KEYWORDS:** Silver Nanoparticles, Antimicrobial, Staphylococcus aureus, Dermal Cream.

INTRODUCTION

Wound infections are one of the bad complications in patients undergoing surgeries. Consequently, infections of different organs or tissues that visible to surgeons may lead to significant increment of postoperative morbidity and mortality beside prolongation of hospital stay.1 It has been well documented that eradication of wound infection resulted in significant increment of patient comfort and decreased medical costs.2 Onche and Adeleji3 stated that *Staphylococcus aureus* is the predominate cause of surgical wound and nosocomial infections. Currently, the antibiotic drug resistance is a fast growing concern in wound infection management beside the risk of impairment of wound healing, bacteraemia, or even sepsis.5 National Nosocomial Infections Surveillance system reported that Methicillin-resistant *Staphylococcus aureus* (MRSA) was responsible for an estimated 94,000 life-threatening infections.5 Silver has been used since ancient times in medical applications as an antimicrobial agent, even before antibiotics were introduced.6 In the last decade silver nanoparticles (AgNPs) have been extensively investigated via *in-vitro* studies to show the antimicrobial activity against bacteria,7–9 fungi10 and viruses.11,12 These studies conclude the high antimicrobial activity of AgNPs against all the tested microorganisms with various degrees. In the last few years, AgNPs invaded the markets in a form of commercial products such as toothpaste, shampoo, water filter, wound dressing and others.13 On the other hand, an obvious cytotoxicity has been reported in experimental rats after chronic or acute dosing of AgNPs.14–17 However the *in-vivo* treatment of infectious diseases is rarely discussed and uncovered. Thus, the balance of beneficial and potential harmful effects is not correctly assessed.

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Also since research work survey not concluded the relative potency of AgNPs in comparison with current topical preparation of antibiotic as fusidic acid (FA). Fusidic acid (FA) is obtained from the fungus *Fusidium coccineum* which was initially found from monkey faeces\(^\text{18}\) and has been available in the market since 1962. The current study aims to investigate the potential antibacterial effect of topical preparation of silver nanoparticles either *in-vitro* or *in-vivo* in comparison with fusidic acid cream.

**MATERIAL AND METHODS**

**Materials**

Silver nitrate, Stearic acid, were purchased from Sigma Aldrich (USA), formaldehyde, potassium hydroxide were purchased from Merck (Germany). Fusidic acid cream (Fucidin, Leo Pharmaceutical Company) obtained from the local market. All reagents were of analytical grade and used without further purification. Double-distilled water was used to prepare all solutions. IgG, IgM and IgA Radial immune diffusion plates were purchased from Biocientifica S.A.

**Synthesis and Characterization of Silver Nanoparticles**

Silver nanoparticles were synthesized by chemical reduction of silver nitrate. In the typical preparation, an aqueous solution of 0.01 M silver nitrate was chilled in an ice bath. After adjusting of pH to 8 by ammonia, a diluted formaldehyde solution was gradually added with vigorous stirring. The mixture turned to yellow which indicated the formation of AgNPs. The UV-Vis absorption spectrum was measured using one mL solution. The solution was freeze-dried to collect AgNPs. The collected AgNPs were washed several times with ethanol, followed by centrifugation (6000 rpm, 10 min). The particles were then dried at room temperature under nitrogen stream.

**In-Vitro Antimicrobial Test of Silver Nanoparticles**

Antimicrobial activity of AgNPs was investigated by well-diffusion method on Mueller-Hinton agar. 9 mL Petri dishes containing sterilized media were separately inoculated with *Staphylococcus aureus* (kindly obtained from Prof. Dr. Azza Abo-Zaid, microbiology lab, Faculty of science, Zagazig University) suspension (10\(^6\) CFU/mL). After media solidification 1 mL diameter wells were punched by sterile gel puncher and immediately filled by AgNPs (1–10 \(\mu\)g/mL) suspension in deionized water under aseptic conditions. The plates were incubated at 37 °C for 24 hours; the antimicrobial activity was assessed by measuring the diameter of inhibition zone around each well.

**Formulation of Dermal Cream Containing Silver Nanoparticles**

In water bath 15 ml of stearic acid was melted then 70 ml of water contain 0.7 g KOH, 15 ml glycerol (2%) w/v AgNPs were added drop wisely. After complete making, the mixture was stirred for 1 min. The formed cream collected and maintained in the refrigerator to be used in next step.

**Animals and Conditions**

Three-months old female Sprague–Dawley rats (120–150) g, purchased from the animal house colony, 6th October, Egypt) were maintained on standard lab diet (protein: 160.4; fat: 36.3; fiber: 41 g/kg and metabolizable energy of 12.08 MJ) purchased from Tanta Feed Co. (Tanta City, Cairo, Egypt). Animals were housed in a room free from any source of chemical contamination, artificially illuminated and thermally controlled at the Animal House Lab., Pharmacology and Chemistry Research Centre, Misr University for Science and Technology (6th October, Giza, Egypt). After an acclimatization period of 1 week, the animals were divided into four groups (10 rats/group) and housed in filter-top polycarbonate cages (five rats per cage). All animals have received human care in compliance with the guidelines of the Animal Care and Use Committee of the Pharmacology and Chemistry Research Centre (6th October City, Giza, Egypt).

**Infection Model**

**Circular Excision Wound Model**

For the evaluation of the antibacterial activity, each rat was undergo anaesthesia by intraperitoneal injection of a combination of 10% ketamine hydrochloride (50 mg/kg) and 2% xylazine hydrochloride (5 mg/kg). The hair of the dorsal back of animals was shaved. For sterilization conditions, the sites of surgery were sanitized by povidone iodine followed by 70% ethanol solution. Circular incision was made on the dorsal inter-scapular region of each animal. After careful skin dissection, the wounds were left...
open, the skin carefully dissected out and the wounds were left open and contaminated locally with $1 \times 10^6$ CFU of *S. aureus* at sites of skin wounds. The bacterial suspension was applied topically for five days throughout the experiment for all animals except the negative control group.

**Experimental Design (Treatment with AgNPs)**

Animals within different treatment groups were treated daily 5 days as follows: (Negative control) untreated group; group (I), animals exposed to *S. aureus* infection for days 5 days; group (II), animals pre-infected with *S. aureus* and treated locally with AgNPs cream for 5 days; group (III) animals pre-infected with *S. aureus* and treated locally with fucidin cream for 5 days.

At the end of the treatment period, blood samples were collected from the retro-orbital venous plexus from each animal under ether anaesthesia. Blood samples were left to clot and the sera were separated using cooling centrifugation at 5,000 rpm for 15 min and stored at $-20 \, ^\circ \text{C}$ until analysis. The sera were used for assessment of Immunoglobulin levels using the radialimmuno diffusion (RID) plates of (Biocientifica S.A. Kit) for IgG, IgM and IgA. After the collection of blood samples, all animals were killed by cervical dislocation and skin samples were collected for histopathological analysis.

**Histopathology**

The skin samples were collected and immediately preserved in the fixation solution (900 ml water, 6.5 g Na$_2$HPO$_4$, 4 g NaH$_2$PO$_4$, 100 ml 40% formalin) and sent to histopathology lab (National Cancer Institute Giza, Egypt).

**Statistical Analysis**

Statistical analysis was performed with SPSS (Version 12). The results were expressed as mean ± SD. Statistical evaluation was performed by analysis of two-tailed Student's *t*-test, *P* values of 0.05 or less were taken as being statistically significant.

**RESULTS AND DISCUSSION**

**Characterization of AgNPs**

The synthesis of AgNPs was confirmed via color change of AgNO$_3$ solution from colorless to yellow and finally dark brown. This solution showed an absorption bands (UV/VIS spectrophotometer, Shimadzu UV1800) at 410 nm (Fig. 1) which is a typical absorption band of spherical AgNPs due to their surface plasmon.$^{19}$ TEM (microscope (JEOL JAM-2100-HR-EM) (National research centre, Giza, Egypt) shows that the particles are monodispersed spherical form with a diameter ranges from 10–20 nm (Fig. 2).

**In Vitro Antimicrobial Activity**

The antimicrobial activity of AgNPs depends on particle’s size and shape, where the antimicrobial activity increases as size decrease.$^{20}$ The prepared AgNPs showed high antimicrobial activity directly proportion with concentration. The MIC was (2 μg/mL).

**General Observation**

Using of safe and effective topical antimicrobial agents is a very important issue in wound healing because the open wounds are at higher risk to infections. The skin of experimental animals which exposed to inoculum for five days were successfully infected as indicated via pus cells and skin lesion. Then the treatment was started immediately with the previously prepared cream or fucidin. The treatment didn’t effect on the mortality or the normal behaviour of the experimental rats. Also there was no any morphological or color change in the skin and no obvious allergy was noted. So, treatment achieved complete cure in about 4–6 days.

<table>
<thead>
<tr>
<th>Serum IgG mg/dl Mean ± SD</th>
<th>Group I N = 10</th>
<th>Group II N = 10</th>
<th>Group III N = 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>3295.47 ± 279.8</td>
<td>1226.9 ± 62.2**</td>
<td>2783.1 ± 147**</td>
<td>1987.8 ± 111</td>
</tr>
<tr>
<td>Serum IgM mg/dl Mean ± SD</td>
<td>115.17 ± 9</td>
<td>68.96 ± 7.56**</td>
<td>277.05 ± 11.89</td>
</tr>
<tr>
<td>Serum IgA mg/dl Mean ± SD</td>
<td>52.25 ± 5.49</td>
<td>51.91 ± 3.5**</td>
<td>65.89 ± 0.7**</td>
</tr>
</tbody>
</table>

Notes: $^*P < 0.05$ Significance as compared with the negative control group. $^{**}P < 0.05$ Significance between group I and II.
Biological Assay
The results of the current study revealed that inoculation with S. aureus, treatment with AgNPs cream or fucidin have significant different effects on humoral immunity in comparison to negative control group as shown in Table I.

Comparing between three treated groups (I, II and III), the result showed significant increase in all the immunoglobulins (IgG, IgM and IgA) concentration level after treatment either by AgNPs cream (group II) or fucidin (group III) as compared with the levels before treatment (group I).

This indicates semi similar positive effect of using AgNPs cream and fucidin on inducing the humoral immunity and ability to stimulate the B cells. Similar results have been reported by Jong et al. In a near view, AgNPs cream showed better results than fucidin, that clear in higher level of serum IgG and lower level of serum IgA that means higher immunity response with lower toxicity respectively. All results are summarized in Table I.

Histopathology
Microscopic examinations Figure 3 of the skin showed that normal morphological appearance of the skin within control group Figure (A). The histological evaluation showed significant changes of structure of the dermal layer beside formation of inflammatory cells in the infected skin Figure (B). Animals treated with AgNPs after five days of inoculum exposure of S. aureus showed significant reduction of the inflammatory cells and succeeded to restore the normal morphological pattern Figure (C). Also fucidin succeeded to reduce the inflammatory cells of the infected skin of the experimental animals (D).

CONCLUSION
In the current study, silver nanoparticles have been synthesized and formulated as topical cream to treat a S. aureus infected wound. The study showed the high significance of using AgNPs for topical treatment of infected wound which indicated via high curing efficiency and no cytotoxicity during and after treatment. Moreover, the study proved that AgNPs cream induce the B cells resulting in a remarkable increase of serum IgG, IgM and IgA levels which is considered as new advantages of our formulation. By comparing with fucidin, they showed almost the same morphological effect but the AgNPs have higher immunological response. This study must be continued with systemic infections to investigate the significance of AgNPs as systemic drug.

REFERENCES


