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RESEARCH ARTICLE

Evaluate the Effect of Biosynthesized Silver Nanoparticles on Wound Healing in Experimental Mice

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Abstract

Biosynthesized silver nanoparticles showed a change in solution color from light yellow to dark brown after 24hr incubation. Results of Ag-NPs characterized by Scanning electron microscopy showed oval, rectangle particles and the average size is 35.1 nm. X-ray diffraction shows one high peak at 2ø (32.5°) as standard XRD data of nano-silver crystals. Antibacterial activity of Ag-Nps against pathogenic isolates *of E. coli*, *P. aeruginosa*, *Klebsiella pneumoniae* and *Staph. aureus* was shown as inhibition zones (11, 12, 14 and 11) mm respectively. There are no toxicity of Ag-Nps in treated mice, and no significant changes nor clinically significant changes observed in the body. Ag-Nps (10mM) showed significant reduction in incision wound for 7 days. Actions of Ag-Nps on wound healing in mice provided a new therapeutic direction for wound treatment in clinical apply.

Keywords: *Biosynthesis of Ag-Nps, E.coli, Antibacterial activity, Wound Healing.*

Introduction

Because of the occurrence of infectious diseases caused by diverse pathogenic bacteria and the development of antibiotic resistance, pharmaceutical companies and researchers are searching for new antibacterial without resistance with low cost [1]. Some organisms such as bacteria, yeast, fungi and algae are able to adsorb and accumulate metal and can be used in the reduction of environmental pollution and also for the recovery of metals from waste [2]. Silver is one of the most powerful antiseptic materials available naturally and non toxic towards mammalian tissue [3].

The most important goal for wound healing accelerated healing without scars for many years, silver sulfadiazine has been the standard treatment for burns, but some of the benefits of the pure silver show to be lost [4]. Wound healing is a complex procedure involving a combination of activities, of different tissues and cell lineages and has been the subject of determining research for a long time [5].

Nanomaterials predictable to affect the medical field in diagnosis, imaging, and drug delivery, because nanoparticles can pass easily by natural barriers, they are used as additives in health related Products such as bandages, catheters, and other materials to avoid infection, particularly through the healing of wounds and burns [6]. Silver nanoparticles (AgNPs) have established the greatest attention due to their wide spectrum of antimicrobial activity such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and klebsiella pneumoniae* [7]. In the present work we investigated biosynthesis of silver nanoparticle from *Escherichia coli*, and evaluated its action on wound healing and toxicity observed in treated mice compared with control groups.

Materials and Methods

Preparation of Bacterial Isolates

The pathogenic bacteria obtained from Biology department, and a Vitek-2 system used to confirm the characterization of isolates.

Synthesis of Ag-Nps using Culture Supernatant of *E.coli* with Glucose

Synthesis of Ag-Nps was achieved by the modified method of [8].

Briefly, bacterial supernatant with (1, 5, and 10) mM of $AgNO_3$ mixed with 1mM glucose in 1:1:1 portion, then the solution was kept in a rotary shaker (200 rpm) at 37° C.

Characterization of Nanoparticles

Scanning Electron Microscopy (SEM)

Type of aggregation and size of Ag-nanoparticles in sample were observed by SEM (TESCAN-VEGA/USA) in Nanocenter/ Technology University with a resolution of 3nm at 30 kv.

Particle Size Distribution Analysis

Zeta plus Particle (Version 5.34), used to establish Ag-nanoparticles size diameter.

Fourier Transform Infra-red Spectroscopy (FTIR)

FT-IR analysis of the Ag-Nps approved by FTIR -8400S (SHIMADZU) at range 4000–600 cm^{-1}.

Stability of Biosynthesized Silver Nanoparticles

This method occupies in flask containing 20 ml silver nanoparticles of each concentration (1, 5, and 10) mM, then kept at 4, 25, and 37c° for more than 7 months, and then stability of color optically observed.

Antibacterial activity by Agar Well Diffusion

The antibacterial activity of the synthesized silver nanoparticles tested against pathogenic bacteria isolates (*E.coli*, *Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumoniae),* which resistant to all kinds of antibiotics. Activity determined by Kirby-Bauer disc diffusion method as described in [9].

Toxicity of Ag- nanoparticles

Selection of Animal Species

Twelve BALB/c male mice, age (8-12) weeks, weight (20-25) gm obtained from Drug Control Center, and kept in animal house in optimal conditions (temperature, 22±3ºC; humidity 30%-60%).

Animals Preparation

The mice acclimatize to the laboratory conditions, for at least five days prior to the start of the study. They were randomly chosen for use in this test, and cages marked to identify 4 groups (each group 3 mice) as: [G1= control (without treatment), G2= mice with 1mM Ag-Nps, G3= mice with 5mM Ag-NPs and G4= mice with 10mM Ag-NPs).

Administration of Doses

Ag-Nps was given (intraperitonealy) at one of the three fixed-dose levels (5, 500, or 2000 mg/kg) to mice. The selection of the initial dose depends on the results of a screening study or from in vitro evidence. The purpose is to identify a dose that produces clear signs of toxicity but no mortality. Depending on the results of the first test, either no further is testing needed or a higher or lower dose is tested: the original dose chosen is 5 mg/kg from concentration (1, 5, and 10) mM. The results were thus interpreted in relation to animal survival and toxicity observation (feature changes, animal's weight loss, etc.) [10].

Wound Healing activity in Vivo

Animal Experiment

Twenty of BALB/c male mice, age (6-8) week, weight (20-25) gm are kept in animal house.

Preparation of Silver Nanoparticles Ointment

Different concentrations of biosynthesized silver nanoparticles (1, 5, 10) mM is mixed and homogenized with 5 gm of the ointment base of each and used for this experiment [11].

Excision Wound Model

Mice were anesthetized by an open mask method with anesthetic ether, they were depilated on the back and determined area about of 0.5 cm, full thickness skin was excised in the saucer. Five groups of mice (each have 4 mice) Group 1= served as a control (untreated), group 2= received ointment, group= 3,4,5 establish different concentrations of silver nanoparticles (1,5, and10) mM respectively. The change in diameter of the wound was measured and expressed as a unit (mm [12].

Results and Discussion

Bacterial Confirmation by Vitek2 System

E.coli isolates have been further confirmed by using a VITEK2 compact system with a gram negative card.

Biosynthesis of Ag-Nps

The color changes in *E.coli* culture supernatant from light yellow to dark brown after 24h of incubation confirmed the synthesis of Ag nanoparticles in the medium (Fig- 1).

Fig. 1: Synthesis of Ag-Nps (a) silver nitrate (b) culture supernatant of *E.coli* (c) mixture of silver nitrate (10 mM) with bacterial supernatant and 100 mM glucose

The strong color change suggests that the synthesis of silver nanoparticles could be better in adding of glucose, [12]. Ref.[13]showed that glucose used as reducing agent because of the encapsulation effect of glucose and trapping the Ag-NPs inside in glucose.

Characterization of Synthesized Silver Nanoparticles

SEM Analysis

(Fig.2) exhibited SEM analysis, and showed less aggregation of Ag-NPs with particles being mostly spherical in shape, and the mean size is about 35.1 nm.

Fig.2: SEM image of Ag- nanoparticles synthesize by *E.coli*

The SEM analysis shows that the synthesized Ag-NPs are oval triangular for *E.coli,* but it appeared spherical for other types of bacteria like *Pseudomonas aeruginosa* [14],*and B. thuringiensis* [15]. SEM is a surface imaging method, fully capable of resolving different particle sizes, size distributions, nanomaterial shapes, and the surface morphology of the synthesized particles at the micro and nanoscales [16].

Particle Size Analysis

Ag-nanoparticles size distributed around (14.1 to 68.1) nm in diameter, (Fig.3).

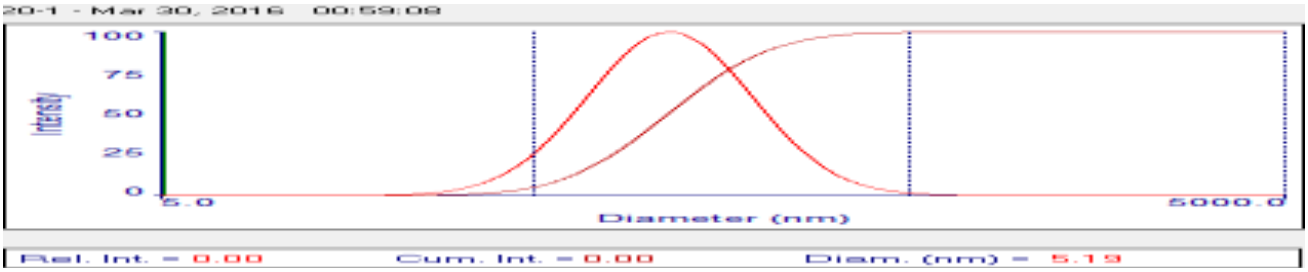

Fig.3: Diameter of S*ilver nanoparticles*

The size of Ag-NPs could be attributed to different cell growth and metal incubation conditions, and the size of which synthesized by *Deinococcus radiodurans* was (25-46) nm [17].

FT-IR Analysis of Ag-Nps

FTIR analysis of Ag-NP (as shown in Figure 4) exposed strong bands at 3361.93 which corresponds to -OH-free, 2927 exemplifies the H-C-H Asymmetric, while band at 2341.58 for O-H stretching corresponds to carboxylic acid, 1367.53 corresponds OH-bend resembling to phenolic compounds can possibly influence the synthesis and stability of AgNPs, whereas the stretch for Ag-NPs found around 518.58 cm-[1].

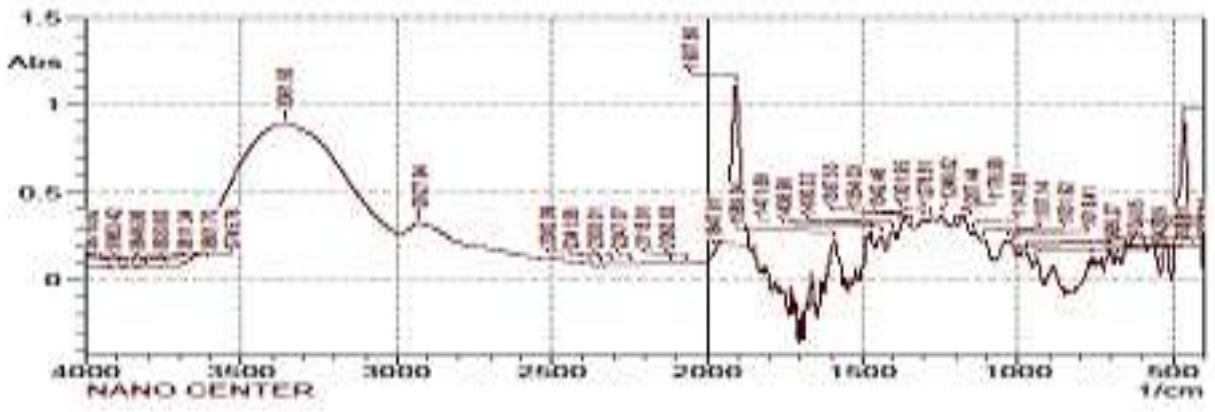

Fig.4: Spectrum of FTIR of silver nanoparticles

FTIR has also been extended to the study of nano-scaled materials, such as confirmation of functional molecules covalently grafted onto silver, carbon nanotubes, graphene and gold nanoparticles, or interactions occurring between enzyme and substrate during the catalytic process [18]. [19]showed that the appearance of the bands at 1668-1679 cm_1 is due to the interactions (mainly Hydrogen bonding) of the silver ions with the -C=O, -NH and –CN stretching modes.

Stability of Biosynthesized Silver Nanoparticles

The synthesized silver nanoparticles formed by *E. coli* remained stable without change in color after incubation in different temperature degrees for 7 months (Fig.5).

Fig. 5: Stability of Ag-Nps after 7month in room temperature

The long-term stability of nanoparticles seriously depends on the medium they are absorbed in [20]. Stable silver nanoparticles in solution are essential to relate and assess their relations with biological matter and living cells 21].

Antibacterial Activity of Silver Nanoparticles

A higher antibacterial effect of Ag-Nps against pathogenic bacterial isolates *E. coli*, *P. aeruginosa*, *Klebsiella pneumoniae* and *Staph. aureus* was observed at concentration of 10µm with inhibition zone of 11, 12, 14 and 11mm, respectively (Figure-6).

Fig. 6: Antibacterial activity of Ag-Nps against *E.coli* by Well diffusion agar

Antimicrobial activity of Ag-NPs are due to the penetration of these particles into the bacteria and damage of the cell membrane and release of cell contents [22]. Silver nanoparticles can react with sulfur-containing proteins inside or outside the cell membrane, and their attack on the respiratory chain, which in turn affect the bacterial cell viability and finally resulting in cell death [23].

The size of the particle plays a central role in antimicrobial activity. The general understanding is that Ag nanoparticle of typically less than 20 nm diameters get attached to sulfur-containing proteins of bacterial cell membranes, leading to greater permeability of the membrane, which causes the death of the bacteria [24].

The colloidal silver particles, with variable sizes (44, 50, 35, and 25 nm), synthesized by the reduction of [Ag (NH3)2]+ complexes with carbohydrates were tested for antimicrobial activity [25]. The antibacterial activity was the particle size dependent.

The silver nanoparticles also exhibit a shape-dependent interaction with the bacterial cells. The truncated triangular silver nanoplates displayed the strongest biocidal action against *E. coli*, when than the spherical and rod-shaped nanoparticles [26]. Small particles exhibited higher antimicrobial activity than big particles. This result can be due to high particle penetration when these particles have smaller sizes. The antibacterial properties are related to the total surface area of the nanoparticles. Smaller particles with larger surface to volume ratios have greater antibacterial activity [27].

Toxicity of Ag- nanoparticles

The results observed in mice was normal for the control and the treated groups, and no significant changes observed in the body weight and no clinical sign change in (skin color, eyes, change in respiration, etc.), also no mortality and toxic signs observed when different doses of colloidal Ag-NPs were injected. Ag-NPs induced diverse degrees of toxicity in vitro depending on nanoparticles size, concentration, and exposure time [28].

In Vivo Wound Healing Activity

The best healing of wound took place in case of mice received (10mM) concentration of silver nanoparticle; full healing obtained within 7 days. The least time of wound healing seen in control group (without ointment and Ag-Nps), which wound failed to heal even after 12th day. The rate of wound healing and concentration of silver nanoparticles showed in (Table-1).

Mice treated with Ag- nanoparticles showed major reduction in wound when compared with control (Fig.7). Ref.[29] explained that silver nanoparticles promoting wound reduction, and play a different role in preventing infection and declining bacterial load in the wound by their broad-spectrum antimicrobial property. Silver nanoparticles also encourage wound healing by modulating cytokine in the wound [11].

Table-1: Percentage of wound contraction in excision wound Model

Groups	Percentage of wound Contraction %	Day of healing wound
Negative Control (Non treated)	11.1%	12
Control (with ointment)	22.2%	11
1mMAgNPs	44.4%	8
5mMAgNPs	77.7%	8
10mMAgNPs	100%	7

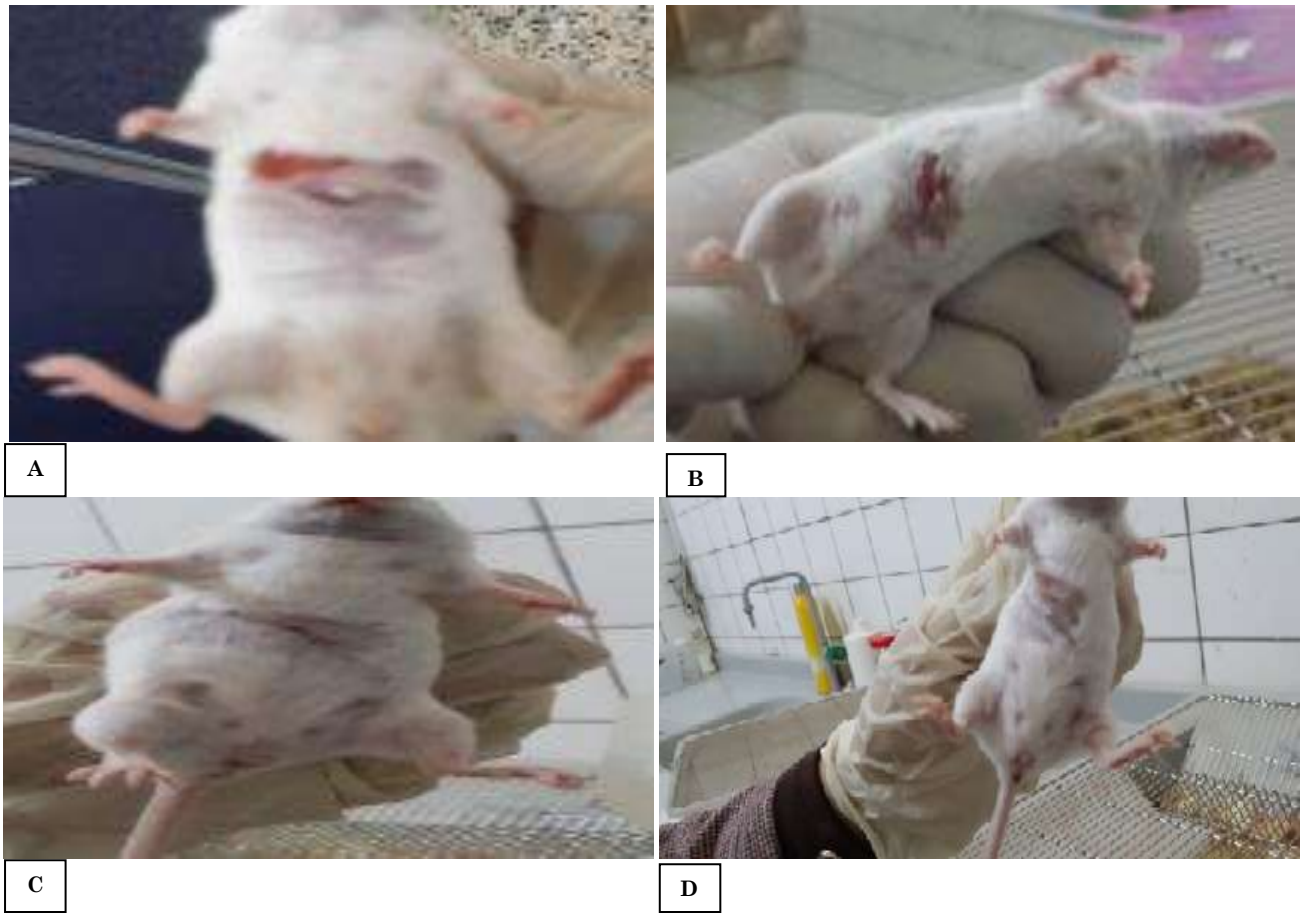

Fig.7: Wound healing (A) Negative control (B) Positive control, (C) Wound after 8 day with Ag-Nps (1mM), (D) Wound after 8 day with Ag-Nps (5mM), (E) Wound Healing after 7 day with Ag-Nps (10mM)

Conclusion

This study aimed to investigate wound-healing properties of biosynthesized Ag-Nps in experimental mice. Silver nanoparticles synthesis by using *E. coli* culture supernatant with glucose showed change in solution color from light yellow to dark brown after 24hr incubation, and it's spherical shape and the average size is 35.1 nm. Biosynthesized Ag-Nps by *E. coli* remained stable after stored for 7 months at different temperatures. Antibacterial activity of Ag-Nps against pathogenic isolates *of E. coli*, *P. aeruginosa*, *Klebsiella pneumoniae* and *Staph. aureus* was observed at concentration of 10µm. There are no toxicity of Ag-Nps in mice, and no significant changes observed in the body weight and no clinical sign change. Rapid healing occurs at dose-dependent manner in mice, and 10mM of Ag-Nps showed significant reduction in excision wound within 7 days.

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