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Comparison of Antibacterial Effect of Biosynthesis Nanoparticles with Chemically Synthesis Nanoparticles in Vitro

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ABSTRACT

The continuation emergence of multidrug resistant bacterial infections and the decline in discovery of new antibiotics are main challenges for health care throughout the world. Recently, chemically and biologically synthesis nanoparticles are used as new antimicrobial agents. Present study focused on detection antimicrobial action of biosynthesis nanoparticles versus chemically synthesis nanoparticles on bacterial infections in Lab. **Method:** in this study, zinc oxide nanoparticles (ZnONPs) and silver nanoparticles (AgNPs) are produced from zinc nitrate and silver nitrate respectively by chemical and biological methods. One concentrations of metallic nanoparticles (60ppm) are tested against *salmonella typhi* (*S. typhi*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Streptococcus pneumonia* (*S. pneumonia*) and *Streptococcus aureus* (*S. aureus*) by disc diffusion method. **Result**: biologically synthesis AgNPs more effected on bacterial species (especially on *S. pneumonia*) than biologically synthesis ZnONPs, chemically synthesis ZnONPs and chemically synthesis AgNPs. On other hand biologically synthesis ZnONPs more effected than chemically synthesis ZnONPs on bacterial species especially *S. pneumonia*. **Conclusion**: biologically synthesis nanoparticles more effected on tested bacterial species especially *S. pneumonia* then chemically synthesis nanoparticles. Biologically synthesis AgNPs are an excellent antimicrobial agent.

Keyword; AgNPs, ZnONPs, salmonella typhi, Pseudomonas aeruginosa, Streptococcus pneumoniae, Streptococcus aureus.

مقارنة التأثير المضاد للبكتيريا لجسيمات النانوية المصنعة حياتيا مع الجسيمات النانوية المصنعة كيميائيا في المختبر

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الخلاصة

استمرار ظهور الاصابات البكتيرية المتعددة المقاومة لأدوية والتراجع في اكتشاف مضادات حياتية جديدة من التحديات الصحية الرئيسية التي تواجة العالم. مؤخرا الجسيمات النانوية المصنعة كيميائيا او حيويا استخدمت كضدات جديدة للجراثيم. الدراسة الحالية ركزت على تحديد التأثير المثبط لجزيئات النانوية المصنعة حياتيا عكس الجزيئات النانوية المصنعة كيميائيا. **طريق العمل**: في هذة الدراسة الجسيمات النانوية لأوكسيد لزنك و الفضة انتجت من نترات الزنك و نترات الفضة بطريقة كيميائية وطريقة حياتية. تركيز واحد(ppm60) من الجسيمات النانوية المعدنية اختبر ضد salmonella typhi (S. typhi), Pseudomonas aeruginosa (P. aeruginosa), Streptococcus pneumonia (S. pneumonia) and Streptococcus aureus (S. aureus) بطريقة الانتشار القرصي. **النتائج:** الجسيمات النانوية للفضة اكثر تأثيرا على الانواع البكتيرية (بالأخص (S. pneumonia) من الجسيمات الناوية للفضة المصنعة كيميائيا و الجسيمات النانوية لأوكسيد الزنك المصنعة كيميائيا او حياتيا. من جهة اخرى الجسيمات النانوية لأوكسيد الزنك المصنعة حياتيا اكثر تأثير على الانواع البكتيرية (بالأخص (S. pneumonia) من الجسيمات النانوية لأوكسيد الزنك المصنعة كيميائيا. **الخاتمة**: الجسيمات النانوية المصنعة بالطريقة الحياتية اكثر تثبيطا لنمو الانواع البكتيرية (بالأخص S. pneumonia) من الجسيمات النانوية المصنعة بالطريقة الكيميائية. الجسيمات النانوية للفضة يمكن اعتبارها مضاد ممتاز للجراثيم .

الكلمات المفتاحية : الجسيمات الفضية النانونية المصنعة، جزيئات اوكسيد الزنك النانونية، الحمى التيفية، المكورات العنقودية السالمونيلا العصوية الكاذبة، المكورات العنقودية الرئوية ، المكورات العنقودية الذهبية.

INTRODUCTION

Drug resistance by pathogenic bacteria remain worldwide problem [1]. Therefore, we require to find out novel strategies or recognize new antimicrobial agents to control microbial infections. Superior effectiveness on resistant strains of microbial pathogens, less toxicity and heat resistance are the characteristic of metal nanoparticles, which make them the selective candidates for eradicating bacteria [2]. Biosynthesis of green nanoparticles using plant extracts is an interesting area in the field of nanotechnology, which has economic and eco-friendly benefits over chemical and physical methods of synthesis [3].

Nanoparticles (NPs) are typically no greater than 100 nm in size and their biocidal effectiveness is suggested to be owing to a combination of their small size and high surface-to-volume ratio, which enable intimate interactions with microbial membranes. In

addition, inorganic antibacterial agents such as metal and metal oxides are advantageous compared to organic compound due to their stability [4]. Among these metal oxides, ZnO has attracted a special attention as antibacterial agent. For instance, ZnO inhibits the adhesion and internal-ization gram negative bacteria. In addition, ZnO nanoparticles exhibit antibacterial activity and can reduce the attachment and viability of microbes on biomedical surfaces [5]. Interestingly, several results suggest a selective toxicity of ZnO-NPs preferentially targeting prokaryotic systems, although killing of cancer cells has also been demonstrated [6]. Several mechanisms have been reported for the antibacterial activity of ZnO-NPs. For example ZnO-NPs can interact with membrane lipids and disorganize the membrane structure, which leads to loss of membrane integrity, malfunction, and finally to bacterial death [7]. ZnO may also penetrate into bacterial cells at a nanoscale level and result in the production of toxic oxygen radicals, which damage DNA, cell membranes or cell proteins, and may finally lead to the inhibition of bacterial growth and eventually to bacterial death [8].

Silver is generally used as nitrate salt, but in the form of Ag nanoparticles (Ag-NPs) the surface area is increased and thereby antimicrobial efficacy is greatly enhanced. Though Ag-NPs find use in many antibacterial applications, the action of this metal on microbes is not fully known. It has been hypothesized that silver nanoparticles can cause cell lysis or growth inhibition via various mechanisms [9,10]. The lethality of silver for bacteria can also be in part explained by thiol-group reactions that inactivate enzymes [11].

Several reports demonstrated the synthesis of ZnO- and Ag-NPs from natural sources like plants or microorganisms by green chemistry approaches [12]. The use of plant extracts for nanoparticles synthe-sis may be advantageous over other biological processes, because it drops the elaborate process of maintain-ing cell cultures and can also be used for large-scale NPs synthesis [13]. Additionally, the green chemistry approach for the synthesis of NPs using plants avoids the generation of toxic byproducts. Among the various known synthesis methods, plant mediated NPs synthesis is preferred as it is cost-effective, eco-friendly and safe for human therapeutic use [14]. In present study, we compared between antibacterial action of biosynthesis nanoparticles and chemically synthesis nanoparticles on some bacterial infections.

MATERIALS AND METHODS

Sample preparation: Different samples collected from patients in Al-Diwaniya teaching hospital. Bacterial species (*S. typhi*, *P. aeruginosa*, *S. pneumonia* and *S. aureus*) are isolated and identified by culture media and biochemical test in bacteriology laboratory in Al-Diwaniya teaching hospital.

Biological synthesis of nanoparticles: *Nerium oleander* leaves have been washed and left to dry at room temperature for 4 days and then sliced into pieces. 10 gram of the leaves have been weighed and placed in a flask with 100 ml distilled water and then boiled for about 5 minutes, filtrated by filter paper and left to chill. 7 ml of the extract were added to an Erlenmeyer flask containing 100 ml of 1mM Silver nitrate or zinc nitrate. The reaction was performed at room temperature and darkness. The reduction of silver and zinc ions was indicated by the transformation of the color into brown which gives us primal evidence of the formation of nanoparticles. A sample of the mixture has been analysed by :Uv -vis spectra, X-ray Diffraction (XRD) , Energy dispersive spectro-scopy (EDS) and Scanning electron microscope (SEM) to characterize the formed nanoparticles.

Chemically synthesis of nano-particles: Chemical reduction method was used for synthesis of nanoparticles by Sodium Boron hydride ($NaBH_4$). To stabilize the solution, 0.3% polyvinyl pyrolidine (PVP) was added to the solution to prevent the particles density. The size of nanoparticles in the zinc or silver nanoparticle suspension were determined by SALD2101. Suspension of zinc or silver nanoparticles became lyophilized powder by freeze-drying method and were kept in a closed container in the refrigerator at 4°C [15].

Bacterial susceptibility to nano-particles: One concentrations (60 ppm) from each of chemically synthesis and biosynthesized nano-particles are tested against *S. typhi*, *P. aeruginosa*, *S. pneumonia* and *S. aureus* in this study. To examine the susceptibility of bacterial species to different nano-particles concentrations, Muller Hinton agar are used. Muller Hinton agar were prepared with holes, the diameter for each hole was 5 mm. In each hole, 0.2 ml of chemically synthesis and biosynthesized nanoparticles are placed (with three replications for each test). Put the plates in the incubator for 24 hrs. in temperature of 37°C then zone of inhibition was measured manually. In additional, dimethyl sulfoxide (DMSO) is placed in other holes as control.

RESULTS

The effect of chemically synthesis and biosynthesized nanoparticles on bacterial species growth can be seen in figure (1). Table (1) showed that AgNPs significantly inhibited

growth of bacterial species ($p < 0.05$) in compared with control. Moreover, biosynthesized AgNPs are more effected on bacterial species than chemically synthesis AgNPs especial on gram positive bacteria (mean of zone of inhibition 31mm and 29mm for *S. pneumonia* and *S. aureus* respectively) as in figure (2). Also, ZnONPs have high antimicrobial effect on bacterial species in compared with chemically synthesis ZnONPs especial on gram positive bacteria (mean of zone of inhibition 28mm and 26mm for *S. pneumonia* and *S. aureus* respectively (figure3). Biosynthesized AgNPs the best inhibitor for bacterial growth (especially *S. pneumonia*) in compared with chemically synthesis AgNPs, chemically synthesis ZnONPs and biosynthesized ZnONPs. In contrast to *S. pneumonia*, *P. aeruginosa* are less effected by nanoparticles (zones of inhibition are 21mm, 20mm, 26mm and 13mm for Biosynthesis AgNPs, Chemo- AgNPs, Bio-ZnONPs and Chemo -ZnONPs respectively)

DISCUSSION

Several approaches have been employed to improve the methods for synthesizing Ag- and ZnO-NPs including chemical and biological methods. Recently, nanoparticles synthesis based on plant extracts is becoming more popular [16,17]. In line with other studies [17,18,24], present study showed that biologically synthesis nanoparticles more active against tested bacterial species then chemically synthesis nanoparticles this may be due to different in properties of produced nanoparticles. The high surface to volume ratio of nano-particles plays an important role in inhibiting the growth of bacteria. Bactericidal effects of nanoparticles is influenced by the particle diameter. Therefore, the choice of synthesis method is effective for controlling the size of nanoparticles [18,19]. The small particles were more antibacterial and had more antibiofilm activity than large particles, as well as, the antimicrobial activity of triangular-shaped nanoparticles more than spherical particles. In the past, studies also reported that antimicrobial activity depends on the size of the nanoparticles [20]. Similar to our data, Doudi *et al.* (2011) and Ruparelia *et al.* (2008) reported that gram negative bacterial species had a higher resistance to silver nanoparticles than gram positive bacterial species. Some researcher believe that lipopoly-saccharide of Gram-negative bacteria trap positively charged silver nano-particles and lead to the blocking of nanoparticles [21,22]. As a result, antibacterial activity of silver nano-particles needs to reach the cell membrane. In fact, the silver nanoparticles are attached to the surface of cell membranes and can disrupted the performance of the membrane, penetrate the cell and release silver ions [21,22,23]. Ghotaslou *et al.*,(2017) showed the effect of silver nanoparticles against *Escherichia* coli was less than *Staphylococcus aureus* and *Pseudomonas aeruginosa* [18]. Salema and his coworkers

(2015) demon-strated that a single oral administration of silver nanoparticles to infant mice colonized with *V. cholerae* or ETEC significantly reduces the colonization rates of the pathogens by 75- or 100-fold, respectively [4]. Furthermore this data agreement with study of Salema his coworkers (2015) who found that AgNPs more effected on bacteria then ZnONPs [4] this may be related to natural, size, shape and other antimicrobial properties of AgNPs compared with ZnONPs [25,26,27]. Oberdorster *et al.*, (2005) demon-strated that the size, shape, surface area, solubility, chemical composition and dispersion factor of nanoparticles play exceptional roles in determining their biological responses [28,29,30].

However, molecular studies are needed to reveal the clear evidence of toxic mechanisms that will be correlated to ZnONPs and AgNPs. Also studies about long-term toxicity, mutagenicity and carcinogenicity are required to clarify any adverse effects of nanoparticles and support the safe use for them.

CONCLUSION

cally synthesis nanoparticles more effected on tested bacterial species especially *S. pneumonia* then chemical synthesis nanoparticles. In contrast to *S. pneumonia*, *P. aeruginosa* are less effected by nanoparticles Biologically synthesis AgNPs are an excellent antimicrobial agent.

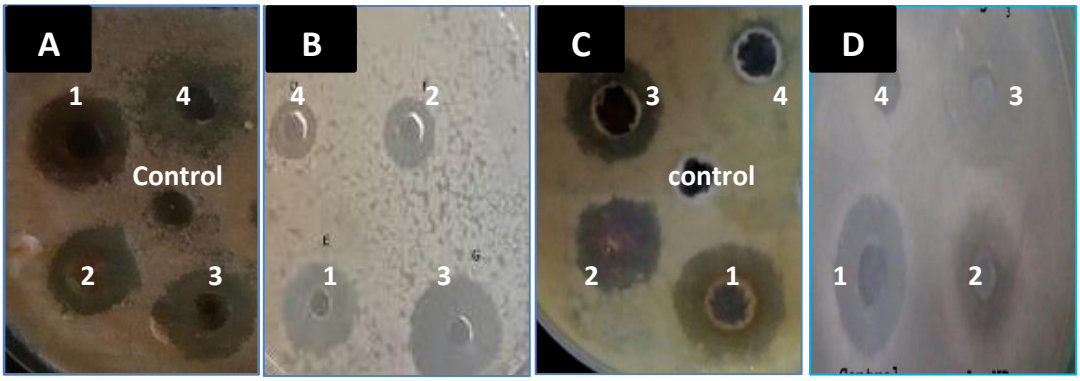

Figure (1): effect of nanoparticles on bacterial species: A= S. *aureus*, B= *P. aeruginosa*, C= *S. pneumonia* and D= *S. typhi* (1= Biosynthesis AgNPs, 2= Chemo-AgNPs, 3= Bio-ZnONPs

Table (1): show bacterial sensitivity to zinc and silver nanoparticles

Bacterial Species	Zone of inhibition (mm) (mean ±SD)				Control (DMSO)
	Biosynthesis -AgNPs (60 ppm)	Chemo-AgNPs (60 ppm)	Bio-ZnONPs (60 ppm)	Chemo ZnONPs (60ppm)	
S. typhi	25±3.88**	19±5.11**	20±1.87**	15±3.09*	0±0
P. aeruginosa	21±5.10**	20±3.211**	26±4.0**	13±6.21*	0±0
S. pneumonia	31±1.99**	22±5.01**	28±1.83**	17±5.74*	0±0
S. aureus	29±5.09**	19±3.64**	26±4.71**	12±1*	0±0

Significant association (p <0.05) in compared with control, **= Significant association (p<0.001) in compared with control, SD = Standard Deviation, NS= Not Significant (p > 0.05).

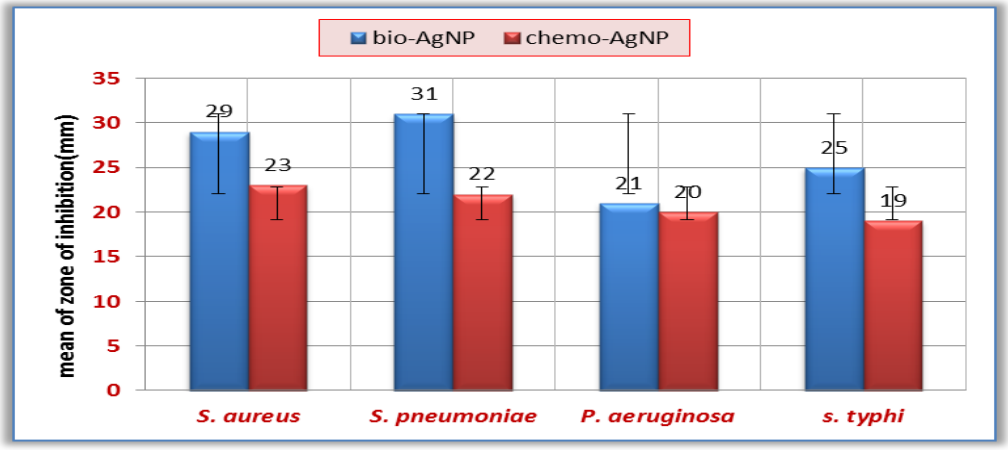

Figure (2): show compared between antimicrobial effect of chemically synthesis and biosynthesized AgNPs on tested bacterial species

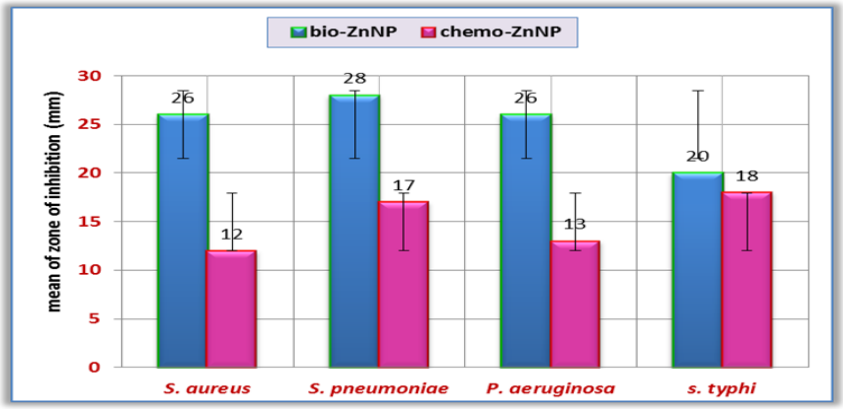

Figure (3): show compared between antimicrobial effect of AgNPs and biosynthesized ZnONP s on tested bacterial species.

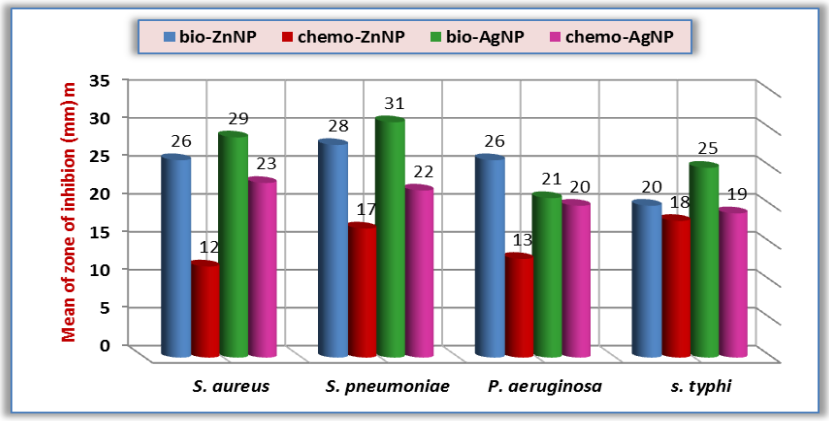

Figure (4): show compared between antimicrobial effect of chemically synthesis and biosynthesized nanoparticles on tested bacterial species.

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