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The use of silver biosynthesized nanoparticles from bacteria *pseudomonas aeruginosa* in the treatment of induced bacterial injury infection

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***Abstract*---**This research, we discuss how biosynthesis silver nanoparticles and used to treat wounds in the skin and compare them with antibiotics and find out which are more effective for healing. at first, nanosilver is synthesis from Pseudomonas aeruginosa it is characterized by confirmation test to proved this material is silver nanoparticles, as well as measuring the susceptibility of the nanomaterial to lysis of red blood cells after that, tests are done for this material in vitro and vivo and that was done by measuring the ability of silver nanoparticles to inhibit the growth of bacteria and comparing it with the inhibition of antibiotics also, the ability of silver nanoparticles to heal was measured on rats at different times and at different doses and compared with the antibiotic.

***Keywords*---**silver nanoparticles, antibiotic, heamolysis assay.

Introduction

The skin composition complex, and includes multifaceted layers responsible for various functions, there are two main components of skin: epidermis and dermis. skin covered with keratin, it is responsible for the physical and biochemical materials functions of the immunohistochemical and adaptive barrier(Proksch *et al* .,2008)

skin damage is a common occurrence in daily life Its effects have been passed over as negligible However, in fact it poses a threat to the security of the whole body, in particular in unhealthy areas, the human body can experience a variety of injuries, including penetration, burns, and blunts shock(Diegelmann, & Evans .,2004) the wound repair steps can be categorized into coagulation inflammation,

proliferation and remodeling with each the stage that involves the cooperative action of many tissues and cells (Nguyen *et al.*,2009) keratinocytes, fibroblasts, endothelial cells macrophages and platelet cells are among the essential components cells are involved in wound repair(Barrientos *et al* .,2008) silver is often used as an antibacterial agent, for Providing a healthy environment for wound healing to treat., historically, silver is considered one of the most used antibacterials before invention of antibiotics(Alexander .,2009) the use of antibiotics contains negative side effects can appear: antibiotic resistance, environmental damage, high costs (Yu *et al.*,2014).

These negative aspects were encouraged by the scientific the community to look for other patterns of bactericidal activity gives a multiplicity of bactericidal mechanisms of silver it has a wide range of effective applications in inhibiting bacteria growth use of broad spectrum antimicrobials silver in the treatment of wounds, to prevent primary growth any bacteria, may contribute to the progression of the wound treatment systems by providing an optimal hygienic environment ment for maximum wound repair, introduction of nanoparticles allowed the scientific community to do so enhances silver's antibacterial properties. growing the surface area of the nanoparticles, in turn, stimulates the increase the rate of interaction between test subjects and ionic silver increased germicidal efficiency reduces the minimum inhibitory concentration, i.e that a lower concentration of the substance may be incorrect classified in wound healing systems to practice the same degree of influence.a lower concentration of silver nanoparticles will reduce potential biological and chemical interference the wound healing processes of the AgNPs themselves in addition, the possibility of modifying nano-silver the molecules increase their beneficial spectrum in the skin reparation this review paper explores recent studies of Provide an overview of the evolution of technologies utilizing silver's antibacterial activity in wound care. (Nam *et al* .,2015)

Material And Method

Antimicrobial activity of sliver nanoparticles

Pre-prepared silver nanoparticles from *Pseudomonas aeruginosa* were used and confirmed by confirmatory tests using three different concentrations (50-100-200µg) For its antimicrobial activity against different types of pathogenic bacteria that have been isolated and diagnosed, including gram-negative and gram-positive bacteria (*staph.aerues, E.coli proteus. Mirabilis, K. pnemumonia*) using well agar diffusion method standardized suspension per tested bacteria (1.5x cfu/ml) by McFarland criterion (0.5N) was scanned separately on muller hinton agar using sterile cotton swabs, the agar was drilled with a sterile cork drill 6 mm, where three were drilled in each dish for a specific type of bacteria used and the three concentrations against each type of bacteria were added to each well. after incubation for 24 hours at 37°C Petri dishes were checked for the diameter of the inhibition zone which was measured by millimeter (Rajeshkumar and Malarkodi, 2014; Skogman *et al.,* 2016)

Susceptibility test of antibiotic

Antibiotics were tested against some types of bacteria in order to compare with the ability of silver nanoparticles against the same types of bacteria in order to obtain the best concentration of silver nanoparticles that are more effective in killing bacteria compared to the antibiotics, the method is as follows:-

1- prepare the Hinton Muller by following the manufacturer's instructions installed on the box and pouring it into Petri dishes.
2- isolate and prepare four types of bacteria used(*staph. aerues ,K.pnemonia ,E .coli ,proteus .mirabilis*).
3- using well agar diffusion method standardized suspension per tested bacteria (1.5x cfu/ml) by McFarland criterion (0.5N) was scanning separately on muller hinton agar using sterile cotton swabs
4- then use antibiotic tablets of four different types (gentamicin, penicillin, rifampin, and tetracycline) and install them in the dishes.
5- We incubate the dishes at 37 °C for 24 hours and then we read the diameter of the areas of inhibition (Fiebelkorn *et al* .,2003)

Heamolysis assay

We did hemolysis tests on one blood healthy donor: 15 µl of nanoparticles (suspended in tyrode), tyrode (negative control) or triton X-100 (positive control) to 285 µl of whole blood, where we have incubated comment at room temperature on a shaking plate during 1 hour 4 hours and 24 hours, after the incubation time, the suspension is centrifuge at 10,000 g over 5 minutes , and then the supernatant is read 96-well plate through the use of scanning microscopic Spectrophotometer at 550 nm(Laloy *et al* ,2014)
The haemolysis was calculated as: H (%) = (OD550nm sample- OD550nm tyrode)/(OD550nm Triton X-100 1% - OD550nm tyrode)*100

Application silver-nanoparticles in vivo
Animal housing

The current study was conducted in the animal house of the College of Veterinary Medicine at Al-Qadisiyah University during the period from (5-19) April 2022, thirty male Wistar rats (90 days old and weight 150 ± 10 g). Male rats were allowed to acclimatize to the animal house environment before starting the experiment .

Expermintal design

Male rats were assigned to (3)equal groups (10 each) and treated as follow:-
Control (C),ointment treated and nano treated :- 10 male rats were shaving with cut of skin and contamination with S.aerues without any treatment in control group but treated ontiment in the second group and nano treated in third group daily for 14 days, male rats were anesthetized (by injection of 0.3ml ketamine + 0.1 ml of xylazine/ kg b.w. ip) and measurement by steel ruler at 0 day , 7 days and 14 days for all groups to compared between it.

Statistical analysis

Statistical analysis Data are presented as mean ± SD. Multiple comparisons were performed using Two-way ANOVA followed by LSD as a post hoc test. The 0.05 level of probability was used as the criterion for significance, all statistical analyses were performed using SPSS software version 27 (Daniel,2009)

Result and Discussion

Antimicrobial activity of sliver nanoparticles

The emergence and increase in the number of many antibiotic- resistant microorganisms, nanoparticles are now considered a viable alternative to antibiotics and appear to have a high potential to solve this problem,in recent years, AgNPs have been considered particularly attractive for the production of a new class of antimicrobials. (Rai *et al.*, 2012)

Antibacterial activity of AgNPs that biosynthesized by *pseudomonas aeruginosa* respectively were used to evaluate their ability for inhibition growth clinical bacteria (table 1) agar well diffusion method was used for detecting the antibacterial activity of nanoparticles using different concentration of AgNPs , the results showed that AgNPs has the ability to inhibit the bacterial growth of both gram positive and gram negative bacteria, the inhibition zone (in millimeter) was greater in gram negative than in gram positive bacteria(table 1).

The results showed when using silver nanoparticles (200 µg) agains *k.peomonia* and *E.coli* bacteria with a significant difference from the rest of the silver nanoparticles concentration (100 and 50 µg), as well as the antibiotics used. the concentration of (200 µg) showed a significant difference from the concentration (100 and 50 µg) in *proteus .mirabilis* and *S.aureus* bacteria, except for the antibiotic penicillin, showed significant difference from all concentrations as well as rifampin,on the other hand, *S. aureus* was more sensitive to the concentration (50 µg) than the other bacteria, and at the concentration (100 µg) the bacteria *k.peumonia, E.coli* showed more sensitivity than the other bacteria, and at the concentration (200 µg) the bacteria *k.peumonia* was more sensitive.

The largest inhibition zone of AgNPs in Gram negative bacteria was(22mm) in *K.pneumoniae* with concentration (200µg/ml),while the largest inhibition zone in Gram positive bacteria was (20mm) in *S. aureus* with same concentration ,in addition the antibacterial activity in gram negative bacteria was also have inhibition zone in their sensitivity to AgNPs when exposed to the same concentration such as *Ecoli ,proteus .mirabales* ,the inhibition zone of these bacteria (20,20 mm) Also it was observed when increased the concentration of AgNPs the inhibition zone increased, (200µg/ml) showed large inhibition zone than 100µg/ml , 50µg/ml respectively (table 1)

The largest inhibition zone was showed in G-ve in comparison with G+ve bacteria, the maximum inhibition zone in G-ve was (22mm) in K.pneumonia with concentration (200µg/ml) of AgNPs while the maximum inhibition zone in G+ve

was (20mm) in S. aureus with the same concentration ,this difference was possibly attributed to the difference of the peptidoglycan layer of the bacterial cell between G+ve and G-ve bacteria.the gram negative cell envelope consists of outer membrane, thin peptidoglycan layer and cell membrane. Beside to this, gram positive cell envelope consists of lipoteichoic acid containing thick peptidoglycan layer and cell membrane(Taglietti *et al.*,2012)

Our result AgNPs synthesized by *pseudomonas aeruginosa* shown the gram positive bacteria *S.aureus* have diameter of the inhibition zone 17 mm, as well as effective against gram negative bacteria *E.coli* was 18 mm with concentration of AgNPs (100 µg) and this result similar to (Oza *et al* .,2012) but the inhibition zone by *K. Pneumonia* is 16 mm and inhibition zone by *proteus .mirebales* is15 mm with concentration (50 µg) agreed with (Maniraj e*t al.*, 2019)

Table (1)
Mean Zone of inhibition (mm) of different concentrations of AgNPs and a number of antibiotics in culture media

Chemical agents	Type of bacteria			
	K.pneumonia	*E. coli*	*Proteus mirabilis*	*S. aureus*
AgNPs 50	16.66±1.15Aa	15±1Ab	15±1Ab	17±1.41Aa
AgNPs 100	18.33±0.57Ba	18.66±1.15Ba	17.66±1.15Ba	17.83±1.47Aa
AgNPs 200	22±1Ca	20±1Cb	20±0Cb	20±0Bb
Penicillin	0±0Da	0±0Da	22.33±0.57Db	0±0Ca
Gentamycin	15.66±0.57Aa	0±0Db	20±0Cc	14±1Da
Tetracycline	0±0Da	0±0Da	0±0Ea	7.5±0.33Eb
Rifampin	0±0Da	0±0Da	10.33±0.23Fb	28±0Fc
LSD(P<0.05)	1.31			

Values represent mean ± SD for 3 batches., capital letters denote to the vertical comparison, small letters denote to the horizontal comparison. Means with different letters in the same column or row are significantly different (P<0.05)

Susceptibility test of antibiotic

Bacterial isolates were obtained from AL-Diwaniyah hospital :three gram negative bacteria (*K.peumonia ,E.coli ,proteus .mirabilis*)and gram positive (*S.aureus*)antibiotic susceptibility test was preformed to detected the effect of some antibiotic on the bacterial isolates and compared with AgNPS by using disc diffusion method –Ddtest (Kirby-Bauer technique) (Hasson.,2018)antibiotic that used against bacterial species in table (1)that showed various susceptibilities to the antibiotic so *E.coli* resistant to gentamycine ,penciline ,tetracycline ,rifampin nearby to (Dogan *et al* .,2012) ,*S.aureus* was sensitive to gentamycine ,tetracycline ,rifadine but resisitant to penciline similar to (Chinnambedu *et al.*,2020)wherese *K. Pneumonia* showed sensitive to gentamycin and resistant penicillin ,tetracycline ,rifampin ,that's result similar to (Schjørring *et al.*,2008) *proteus .mirabilis* sensitive to penciline ,gentamycin ,rifampin and resistant to tetracycline (Chanyi *et al.*,2018)

Plasmids and transposons are the most important mobile genetic elements (MGEs) that play a critical role in the development and dissemination of antimicrobial resistance to many pathogenic organisms(Bozcal .,2019) integrons also provide a powerful strategy and efficient gene exchange mechanism since it is easy to add new genes to the bacterial chromosome and in addition to their ability to provide the necessary machinery to ensure their expression, are among the most important drivers of bacterial evolution(Gillings.,2014)

Heamolysis assay

Hemolysis is the process of breaking down platelets, when AgNPs are injected into the blood for drug delivery and the harmful cooperation of these nanoparticles with blood components should be avoided. thus the hemolytic effect of erythrocytes was tested with different concentrations of AgNPs (512,256, 128, 64 µg/ml), respectively, in PBS and the hemolytic properties that vary with the concentration of AgNPs, a low hemolysis of 1.18% was observed with 64 µg/ml of AgNPs. according to the american society for testing and materials, the tests were deemed safe because the hemolytic rate was less than 5%. in our experiments, the estimated hematologically compatible properties of AgNPs indicated <5% at prominent concentration of AgNPs like the result in(Vanaraj *et al* ., 2017)

Application silver-nanoparticles in vivo

Three groups of male laboratory rats were used, the control group, the ointment group and the silver nano group, where a 2-cm wound was made on all rats and contaminated with bacteria *Staphylococcus aureus* and they were treated at three intervals of(one day, 7 days and 14 days), the results shown there was no significant difference ($p > 0.05$) between the three groups on the first day, but the results showed a significant difference of the AgNPs group from the rest of the treatments on the seventh and fourteenth day, and there was a significant difference ($p < 0.05$) between the three groups and between the three times shown in the table(2)and our result agreed with (Gong *et al.*, 2018) the wound closure time was less in treated with silver nano group with compared to another groups(Odimegwu *et al.,* 2008)

as wound has little breaking strength in the beginning, that increases rapidly during healing due to the synthesis of collagen and formation of intra and intermolecular cross linking. here, an increase in skin breaking strength of the animals treated with the AgNPs based ointment formulations explained that the active biomolecules present in it were assisted in the enhanced synthesis of aldehyde groups of collagen fibres for cross linkage of the skin in the rats(Bhuvaneswari *et al.*, 2014)

Table (2)
Wound diameter (cm) in the rats of different groups

Groups	Period		
	1 day	*7 days*	*14 days*
Control	2.11±0.02Aa	1.48±0.05Ab	1.12±0.01Ac
Ointment	2.11±0.02Aa	1.19±0.03Bb	0.82±0.02Bc

AgNPs	2.11±0.02Aa	1.01±0.04Cb	0.36±0.03Cc
LSD(P<0.05)	0.030		

Values represent mean ± SD for 3 batches., capital letters denote to the vertical comparison, small letters denote to the horizontal comparison. Means with different letters in the same column or row are significantly different (P<0.05)

Conclusion

biosynthesized AgNPs from *pseudomonas aeruginosa* demonstrated potency antimicrobial activity against many pathogens that have been it was tested in the current study, the use of AgNPs in the possibility of wound healing in male rats by using it in wounds contaminated with bacteria and measuring the duration of wound healing, AgNPs also promoted wound healing activity in rats , on the basis of previous work and research in this field, it is possible to conclude that biosynthesis AgNPs can be considered as cost-effective, anti-hemolytic it is an effective therapeutic agent to control the growth of bacteria

Reference

Alexander, J. W. (2009). History of the medical use of silver. Surgical infections, 10(3), 289-292

Barrientos, S., Stojadinovic, O., Golinko, M. S., Brem, H., & Tomic-Canic, M. (2008). Growth factors and cytokines in wound healing. Wound repair and regeneration, 16(5), 585-601

Bhuvaneswari, T., Thiyagarajan, M., Geetha, N., & Venkatachalam, P. (2014). Bioactive compound loaded stable silver nanoparticle synthesis from microwave irradiated aqueous extracellular leaf extracts of Naringi crenulata and its wound healing activity in experimental rat model. Acta Tropica, 135, 55-61.

Bozcal, E. (2019). Insight into the mobilome of Escherichia coli. In The universe of Escherichia coli. IntechOpen

Chanyi, R. M., Alzubaidi, R., Leung, E. J., Wilcox, H. B., Brock, G. B., & Burton, J. P. (2018). Inflatable penile prostheses implantation: does antibiotic exposure matter?. Sexual Medicine, 6(3), 248-254

Chinnambedu, R. S., Marimuthu, R. R., Sunil, S. S., Amrose, P., Ramachandran, V., & Pachamuthu, B. (2020). Changing antibiotic resistance profile of Staphylococcus aureus isolated from HIV patients (2012–2017) in Southern India. Journal of Infection and Public Health, 13(1), 75-79

Daniel, W. (2009). Biostatistics: A Foundation for Analysis in the Health Sciences. 9th edition. John Wiley and Sons. INC. USA

Diegelmann, R. F., & Evans, M. C. (2004). Wound healing: an overview of acute, fibrotic and delayed healing. Front biosci, 9(1), 283-289

Dogan, B., Scherl, E., Bosworth, B., Yantiss, R., Altier, C., McDonough, P. L., ... & Simpson, K. W. (2012). Multidrug resistance is common in Escherichia coli associated with ileal Crohn's disease. Inflammatory bowel diseases

Fiebelkorn, K. R., Crawford, S. A., McElmeel, M. L., & Jorgensen, J. H. (2003). Practical disk diffusion method for detection of inducible clindamycin resistance in Staphylococcus aureus and coagulase-negative staphylococci. Journal of clinical microbiology, 41(10), 4740-4744 .

Gillings, M. R. (2014). Integrons: past, present, and future. Microbiology and molecular biology reviews, 78(2), 257-277

Gong, C. P., Li, S. C., & Wang, R. Y. (2018). Development of biosynthesized silver nanoparticles based formulation for treating wounds during nursing care in hospitals. Journal of Photochemistry and Photobiology B: Biology, 183, 137-141

Hasson, S. O. (2018). Effect of biologically and chemically synthesized silver nanoparticles on biofilm forming bacteria isolated from catheterized Iraqi patients (Doctoral dissertation, Ministry of Higher Education)

Laloy, J., Minet, V., Alpan, L., Mullier, F., Beken, S., Toussaint, O., ... & Dogné, J. M. (2014). Impact of silver nanoparticles on haemolysis, platelet function and coagulation. Nanobiomedicine, 1, 4

Maniraj, A., Kannan, M., Rajarathinam, K., Vivekanandhan, S., & Muthuramkumar, S. (2019). Green synthesis of silver nanoparticles and their effective utilization in fabricating functional surface for antibacterial activity against multi-drug resistant Proteus mirabilis. Journal of Cluster Science, 30(6), 1403-1414

Nam, G., Rangasamy, S., Purushothaman, B., & Song, J. M. (2015). The application of bactericidal silver nanoparticles in wound treatment. Nanomaterials and Nanotechnology, 5(Godište 2015), 5-23

Nguyen, D. T., Orgill, D. P., & Murphy, G. F. (2009). The pathophysiologic basis for wound healing and cutaneous regeneration. In Biomaterials for treating skin loss (pp. 25-57). Woodhead Publishing

Odimegwu, D. C., Ibezim, E. C., Esimone, C. O., Nworu, C. S., & Okoye, F. B. C. (2008). Wound healing and antibacterial activities of the extract of Dissotis theifolia (Melastomataceae) stem formulated in a simple ointment base. Journal of medicinal plants research, 2(1), 011-016

Oza, G., Pandey, S., Shah, R., & Sharon, M. (2012). Extracellular fabrication of silver nanoparticles using Pseudomonas aeruginosa and its antimicrobial assay. Pelagia Res Lib Adv Appl Sci Res, 3(3), 1778-1783

Proksch, E., Brandner, J. M., & Jensen, J. M. (2008). The skin: an indispensable barrier. Experimental dermatology, 17(12), 1063-1072

Rai, M.K.; Deshmukh, S.D.; Ingle, A.P. and Gade, A.K. (2012). Silver nanoparticles: The powerful nanoweapon against multidrug-resistant bacteria. J. Appl. Microbiol., 112: 841–852

Rajeshkumar, S., & Malarkodi, C. (2014). In vitro antibacterial activity and mechanism of silver nanoparticles against foodborne pathogens. Bioinorganic chemistry and applications, 2014

Schjørring, S., Struve, C., & Krogfelt, K. A. (2008). Transfer of antimicrobial resistance plasmids from Klebsiella pneumoniae to Escherichia coli in the mouse intestine. Journal of Antimicrobial Chemotherapy, 62(5), 1086-1093

Siritongsuk, P., Hongsing, N., Thammawithan, S., Daduang, S., Klaynongsruang, S., Tuanyok, A., & Patramanon, R. (2016). Two-phase bactericidal mechanism of silver nanoparticles against Burkholderia pseudomallei. PloS one, 11(12), e0168098

Skogman, M. E., Vuorela, P. M., & Fallarero, A. (2016). A platform of anti-biofilm assays suited to the exploration of natural compound libraries. JoVE (Journal of Visualized Experiments), (118), e54829

Taglietti, A., Diaz Fernandez, Y. A., Amato, E., Cucca, L., Dacarro, G., Grisoli, P., ... & Patrini, M. (2012). Antibacterial activity of glutathione-coated silver

nanoparticles against gram positive and gram negative bacteria. Langmuir, 28(21), 8140-8148

Vanaraj, S., Keerthana, B. B., & Preethi, K. (2017). Biosynthesis, characterization of silver nanoparticles using quercetin from Clitoria ternatea L to enhance toxicity against bacterial biofilm. Journal of Inorganic and Organometallic Polymers and Materials, 27(5), 1412-1422

Wu, D., Fan, W., Kishen, A., Gutmann, J. L., & Fan, B. (2014). Evaluation of the antibacterial efficacy of silver nanoparticles against Enterococcus faecalis biofilm. Journal of endodontics, 40(2), 285-290

Yu, L., Zhang, Y., Zhang, B., & Liu, J. (2014). Enhanced antibacterial activity of silver nanoparticles/halloysite nanotubes/graphene nanocomposites with sandwich-like structure. Scientific reports, 4(1), 1-5