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The use of silver nanoparticles synthesized by *Proteus mirabilis* in treating of induced bacterial burn infections

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***Abstract*---**Burn infections pose a major health challenge due to the emergence of bacterial strains that are resistant to antibiotics, which prompted scientists to find other more effective materials, and that the bio-manufactured silver nanoparticles proved effective against the bacteria that cause infections burns such as (*Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia and Escherichia coli*) This study was conducted to assess the activity of silver nanoparticles (AgNPs) synthesized by *Proteus mirabilis* to promote the healing of burns in rats was used 30 animals in the experiment, all suffered burns a diameter of 2.2 cm using caustic electrical and divided into three groups each total of 10 animals first group control without any treatment, the second group treatment with traditional ointments such as tetracycline, the third group treatment with nanoparticles were evaluated wound healing by measuring the diameter burn in 1, 7 and 14 days with treatment daily, the results of this study showed that the nanoparticles gave better results when compared to the traditional ointment, as well as knowledge effectivel nanoparticles against types of bacteria that cause infections burns through the diameter measuring inhibition of bacterial growth, and identify minimum inhibitory concentration for the growth of micro-organisms.

***Keywords*---**burn infections, silver nanoparticles, treating, wound healing.

Introduction

Healing burn wounds and regenerating damaged skin tissue continue to be two of the most significant obstacles that humanity must overcome, especially

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considering the prevalence of bacterial infections among burn patients (Mundhada *et al.*,2015). In addition to *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia and Escherichia coli* is recognized as a prominent colonizer of burn wounds.(Cato *et al.*, 2021). Because of its capacity to spread quickly in hospital settings and its resistance to antibiotics, recent years have seen an increase in both morbidity and fatality rates.(Chan *et al* .,2010 , Yu *et al.*,2015). Because of multidrug-resistant bacteria and the difficulties of producing new antibiotics, new types of treatments such as phage therapy must be explored. (Gondil,*et al.*, 2018) enzymbiotics, antibacterial nanoparticles(Kumar*, et al* .,2017)

Size, geometry, surface changes, and technique of synthesis affect silver nanoparticles' antibacterial activity. Silver nanoparticles are antibacterial through free radical production, membrane rupture, DNA destruction, and DNA replication inhibition. (Chhibber,et al ., 2017). Topically applied silver nanoparticles have been examined as a technique to deliver an adequate dosage while reducing side effects and systemic exposure, resulting in fewer unwanted consequences. (Ghorbani,*et al.*, 2018). AgNP is a broad-spectrum antibacterial Antimicrobial characteristics and antibacterial effectiveness (Sharma *et al.,*2009). The effectiveness of Nano particles on bacterial activity and a topical delivery system silver nanoparticles in treating *Staphylococcus aureus* –induced murine burn wound infection was investigated in this study.

Materials and Methods

Concentrations of AgNPs and MIC-AgNPs

the minimum inhibitory concentration (MIC), as well as the minimum bactericidal concentration (MBC), that were employed in this study were based on a previous work that was conducted (Chhibber et al .,2017)
All isolates' MICs were obtained using serial dilutions.

1 every micro plate test tube received volume of 100 μL brain heart infusion broth.
2 using a sterile wood stick, numerous colonies were transferred from plates to a saline tube to make a homogenous organism solution.
3 the suspension was adjusted to 0.5McF using a dennis check plus meter.
4 pipette 100 μL AgNps (512 μg/ml) into column 1 wells.
5 using a 100 μL micropipette, mix the tested solution into column 1 wells 6-8 times. Don't splat
6 Add 100 μL to column 2 from column 1. Column 2 dilutes column 1 twice. 100 μL into column 3. Rep till just column 11 is reached.
7 Discard 100 μL from column 11, not column 12.
8 Using a 5 μL micropipette, distribute bacteria into all wells except column 12. (sterility control and blank for the plate scanner).
9 The micro titer plates were incubated at 37°C for 24 hours after inoculation. Recording OD600 nm Changes in ODs over NP concentration were utilized to produce growth inhibition curves after 24 h. .(Khan *et al.,* 2010 ; Kumur *et al.*,2015)

Antibacterial activity of silver nanoparticles

Using agar well diffusion, AgNPs biosynthesized by *proteus mirabilis* were evaluated for antibacterial efficacy against Gram positive and Gram negative clinical bacteria (*Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia and Escherichia coli*). Each tested microorganism was swabbed separately onto sterile Brain Heart Agar (BHA) plates using sterile cotton swabs (1.5x cfu/ml). 100 µg / ml of biogenic AgNPs from *proteus mirabilis* were put to each well of sterilized 6 mm agar. Incubation lasted 24 hours. After 37°C incubation, millimeter-diameter inhibitory zones were detected (Rajeshkumar and Malarkodi,2014).

Treatment of induced burns in laboratory animals by nanoparticles

The present study has been conducted in the animal house of the college of veterinary medicine, AL-Qadisiya University during the period extended from (5^{th}-19^{th}) March, 2022. Thirty mature male Wister rats with ages of 90 days and weights of 150 +/- 10 g each were used in this experiment. Before beginning the experiment, it was decided to give the male rats time to become accustomed to the conditions of the animal house. Within a chamber that had enough ventilation, the animals were kept in cages made of polypropylene. The maximum number of rats that can be found in any one cage is five. Throughout the entirety of the trial, male rats were given the regular diet and allowed to drink water at their own leisure. During the course of the experiments, the room temperature was kept at 23.2 degrees Celsius, and the light and dark cycle was set to alternate every 12 hours, with the lights turning on at 6:00 a.m. and turning off at 6:00 p.m..
Experimental design:
Male rats were assigned to 3 equal groups (10 each) and treated as follow:

1. Control (C):- 10 male rats were shaving with burn of skin by electric treadmill without any treatment.
2. Ointment treated :- 10 male rats were shaving with burn of skin by electric treadmill with ointment daily for 14 days.
3. Nano treated :- 10 male rats were shaving with burn of skin by electric treadmill with Nano daily for 14 days.

Throughout the course of the experiments, male rats have been observed for monitoring purposes. At the conclusion of each treatment and control period, male rats were rendered unconscious through intra peritoneal injection of 0.3 milliliters of ketamine and 0.1 milliliters of xylocain per kilogram of body weight. In order for all of the groups to be able to compare their results, measurements were taken using a steel ruler at 0 days, 7 days, and 14 days.

Result

Determination of Minimum Inhibitory Concentration (MIC) of silver nanoparticles

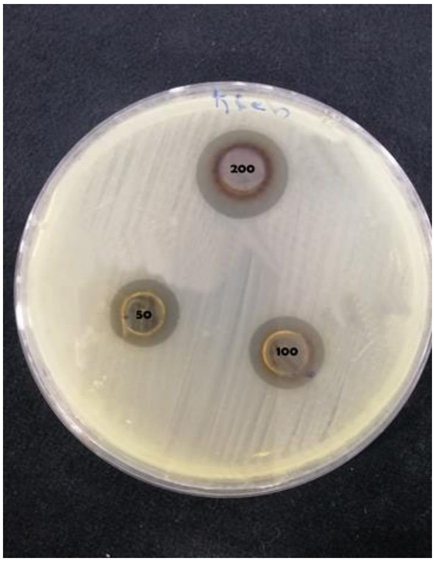
P. aeruginosa, S. aureus,E coli and K. pneumonia were treated with increasing concentrations of AgNPs (1, 2, 4, 8, 16, 32, 64, 128, 256, 512 μg/ml) and incubated for 24 h at 37 C. AgNPs suppress bacteria dose-dependently, according to our results. AgNPs have MICs of 32μg/mL against *P. aeruginosa and K. pneumonia* and 16 μg/mL against *S. aureus* and *E. coli.*(Khan *et al.,* 2010)

Antibacterial activity of silver nanoparticles

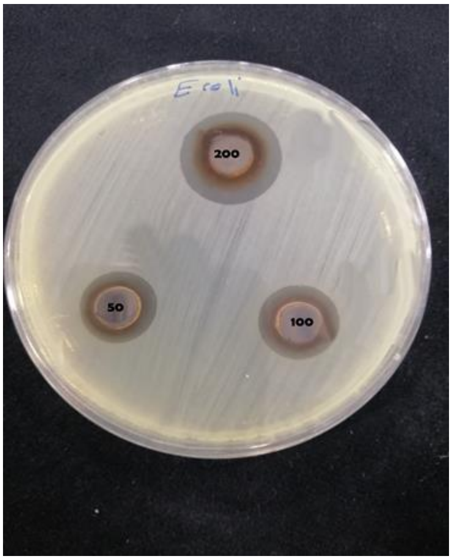
Three concentrations (50, 100, 200) μg / ml of SNPs were tested against bacteria in the present investigation using well diffusion method; to determine the concentration that display broader zone of inhibition, as shown in figure (4-20). 200 ug/ml SNPs produced a wider inhibitory zone than 50 and 100 ug/ml.

Table (1)
Diameter of bacterial growth inhibition according to AgNPs concentration

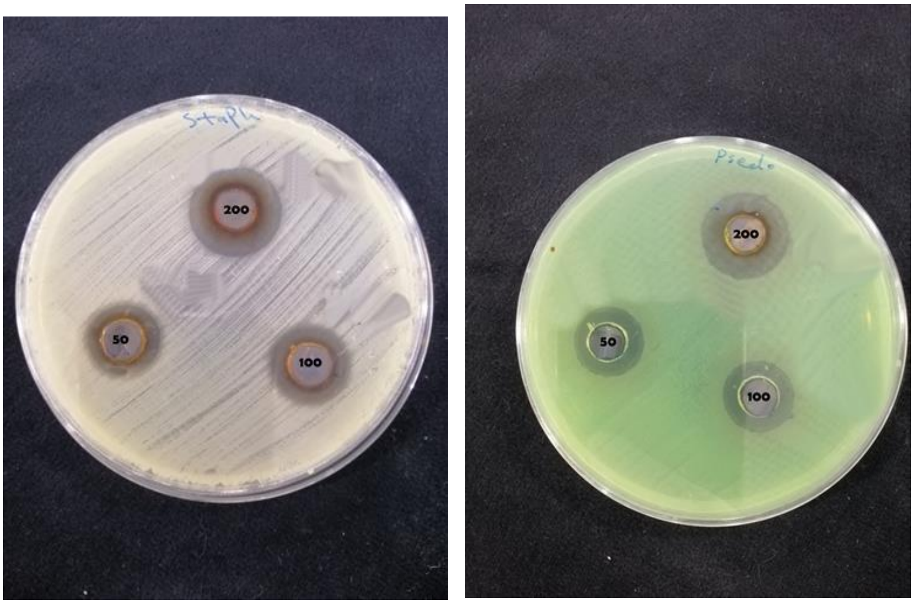
Type of bacteria	Diameter at 200 µg / ml of SNPs	Diameter at 100 µg / ml of SNPs	Diameter at 50 µg / ml of SNPs
E. coli	22 mm	18 mm	16 mm
P. aeruginosa	21 mm	17 mm	15 mm
S. aureus	20 mm	18 mm	16 mm
K. pneumoniae	20 mm	17 mm	15 mm



(A) *K. pneumonia*



(B) *E coli*



(C) *S. aureus* (D) *P. aeruginosa*

Figure (1) Diameter of bacterial growth inhibition according to AgNPs concentration

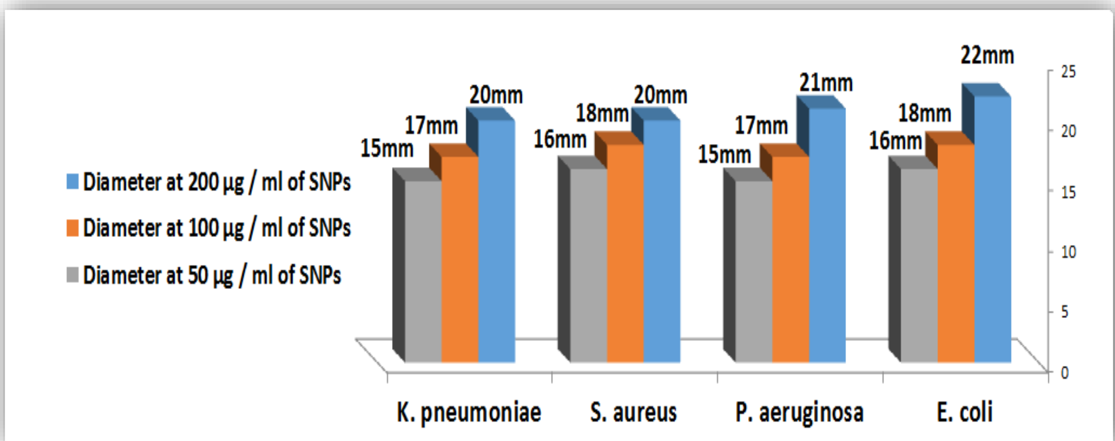


Fig (2) The antibacterial activity of AgNps at different concentration

Treatment of induced burns on laboratory animals by biosynthetic nanoparticles

In his experiment, we focus on two factors first factor is treatment time (one day, 7 days and 14 days). The second factor is treatment type (Without treatment, Ointment treatment and Nano treatment). Results were expressed as mean ± standard error (SE). Comparisons were performed using two way analysis of variance (ANOVA2) and Least Significant Difference (LSD) test all groups unpaired values. As following table:

Table (2)
Show effecting of treatment time and treatment type on Healing from burns

treatment time	treatment type	Mean± S.E	Statistical Decision
One day	Control (without treatment) (A)	2.216 ± 0.0051^{AA}	No significant difference
	Ointment treatment (B)	2.186 ± 0.0050^{AA}	No significant difference
	Nano treatment (C)	2.213 ± 0.0038^{AB}	Significant difference
7 days	Control (without treatment) (A)	1.612 ± 0.0059^{AA}	No significant difference
	Ointment treatment (B)	1.198 ± 0.0059^{AB}	Significant difference
	Nano treatment (C)	0.903 ± 0.0126^{AB}	Significant difference
14 days	Control (without treatment) (A)	1.073 ± 0.0037^{AB}	Significant difference
	Ointment treatment (B)	0.712 ± 0.0037^{AB}	Significant difference
	Nano treatment (C)	0.347 ± 0.0204^{AB}	Significant difference
F_C= 1194.592 **P=0.** 000	**F_C=**11889.526 **P=0.** 000		

***F_C** is represented F calculated *Different letters denote to significant difference (**P**< 0.05). Similar letters denote to non-significant difference (**P**> 0.05).

From the results shown in the table (2) the first factor is the time of treatment, where it was found that there was a clear difference in the diameter of the burn when it was measured in (1,7,14) days between the three groups A,B,C, meaning that there is a relationship between the diameter of the burn and the treatment time. The second factor influencing is the type of treatment where he found a variation in the speed of healing wounds between the three groups by user treatment as the type found, that the group (C) that was treated by Nanoparticles (100 μg/ml) faster healing than other groups , the group treated by the traditional ointment(B) faster healing than the first group (A). This means the our Nano treatment is more significant on burn healing compared with other treatment type. The following figure show our Nano treatment is very active.

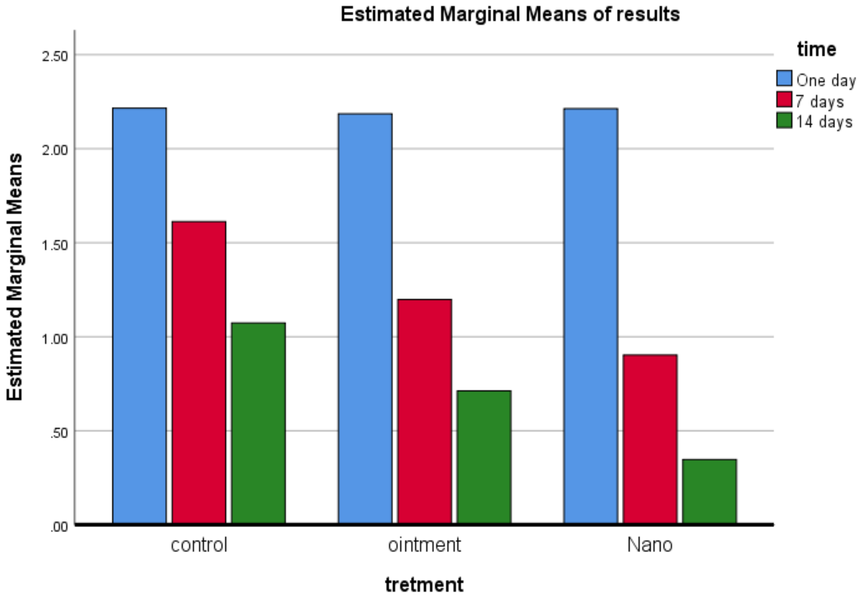


Fig (3) the mean of treatment type and treatment time in burn healing

All results were carried out using the spss (Spss Institute, Inc., USA).

Discussion
Determination of Minimum Inhibitory Concentration (MIC) of silver nanoparticles

AgNPs have MICs of 32μg/mL against *P. aeruginosa and K. pneumonia* and 16 μg/mL against *S. aureus* and *E. coli*. These results consistent with Veerapandian *et al* (2010) It should be noted that Gram-negative bacteria MICs ranged from between(16 - 64μg/ml) ,When compared to Gram-positive bacteria (16-128μg/ml) and other types of bacteria.

Antibacterial activity of silver nanoparticles

The present study used Three concentrations (50, 100, 200) μg / ml of SNPs were tested against bacteria((*Staphylococcus aureus , Pseudomonas aeruginosa, Klebsiella pneumonia and Escherichia coli*) in the present investigation using well diffusion method; to determine the concentration that display broader zone of inhibition, as shown in figure (1). The concentration 200 μg/ml generated a broader zone of inhibition, but the concentrations (50, 100) μg/ml of SNPs are less inhibition zone. This result suggests that increasing the concentration of SNPs increases antibacterial efficacy. Yamamoto *et al.* (2004) employed 200 μg/ml silver nanoparticle-saturated filter paper. This study found a substantial increase in inhibitory efficacy (P 0.05) when SNPs were used at a dose of 200 against all bacteria tested, compared to all antibiotics examined.

It was consistent with Willing *et al* (2018) study that found inhibition of both gram (+) and gram (-) by Three concentrations (50,100, 150) µg/ml of SNPs were tested against the bacteria. The other study found that silver nanoparticles produced from *Proteus mirabilis* showed the greatest zone of inhibition against a variety of harmful pathogens, including *Staphylococcus aureus* and *Pseudomonas SPP*. (Karthick *et al* .,2013)

Treatment of induced burns on laboratory animals by biosynthetic nanoparticles

Through the results we obtained, it was found that the rate of burns wound healing of animals treated with nanomaterials is faster than the group that was treated with traditional treatments. And that the group treated with the traditional ointment healed faster than the non-treated group, and this means that the nanomaterial used in treating burns is of excellent efficacy. These results were identical to what his Pannerselvam *et al* (2017) In the sub epidermal layer of the tissues of the experimental animals that were given 30 or 50 micrograms of AgNPs, there was no evidence of fibroid necrosis. The findings of our research are consistent with those of other studies which found that the use of various therapeutic forms of nano crystalline silver particles accelerated wound healing (Ayuningtyas et al 2021; Veshnavei ,2021)

Conclusion

The present study has shown effective nanoparticles bio synthesized effectiveness against the bacteria that cause infections of burns and also gave positive results in the speed of wound healing when compared with traditional ointments used in the treatment of burns, such as tetracycline, was reached minimum inhibitory concentration (MIC) where found between (16 - 32 μg / ml)depending on the type of bacteria

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