RESEARCH ARTICLE

The effect of Silver Nanoparticle Induced Diabetic on Wound Healing Full Thickness *Pseudomonas aeruginosa* Contaminated Mouse Skin Wound Models

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ABSTRACT

This research was considered to evaluate the antidiabetic effect of silver nanoparticle (AgNps)and following experimental diabetic. In the present study. Thirty healthy swiss mice aged between 7–8 weeks, old male mice and divided into six groups of five animals. Diabetic induced mice by using intraperitoneal (IP) injection of alloxan (180 mg lkg). Group 1 included non diabetic control, Group 2 Diabetic, Group 3 Diabetic +0.01 mg AgNps Group 4 Diabetic +0.05 AgNps, Group 5 Diabetic+ wound contaminated with *Pseudomonas aeruginosa*. Group 6 (diabetic +contaminated wound + silver nanoparticle (Ag Nps). Silver nanoparticle show ample antibacterial activities. The result of the current study introduced an *invivo* silvernanoparticle accelerate by effects on the treatment of *Pseudomonas aeruginosa* infected skin wound. The present study was conducted to synthesis the AgNps biologically and evaluate its antibacterial activity against *Pseudomonas aeruginosa* diabetes induced by Alloxan in mice. Adminstration of silver nanoparticle resulted in significance antidiabetic effects that is is improved glucose tolerance higher source. The current study results are presented for the first time which suggest for the development of AgNps as anantdiabetic factor in future. The broad spectrum of bioactivity of AgNPs makes them promising agent not only to fight infection, but to sterile the wound and accelerate wound healing. There were significant higher wound healing scores in Nanoparticle treated group. Compared with control group. These result suggest that nanoparticle may be useful in diabetic wound healing. Treatment with asingle dose of AgNPs produced amild reduction in blood glucose and some reduction in plasma insulin at 2 h.The present results revealed the potential of the synthesized Ag-NPs as safer bactericidal agents for the treatment of diabetes induced wound contaminated with P.aeruginosa.

Keywords: Alloxan, Antibacterial, Diabetes, *Pseudomonas aeruginosa*, Silver nanoparticle. Wound healing.

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INTRODUCTION

The use of nanotechnology in medicine as therapy for diabetes mellitus has been applied largly,[1] through developing may antidiabetic agents for controlling diabetes using *invitro* and *invivo* research method some of these agent are restricted due to their pharmacokinetic possession.[2] Has potential.Nanomaterial ametabolic disorders are developing field of nanotechnology drug increasing important to their unusual optical, chemical, photoelectron chemical.[3] Nanotechnosience[4] play an increasing cruicallyl role in many key technology of the new mellenium. It is gainig important Increase such as optic, biomedical science.[3] Diabetic is ametabolic disorder that result from disease insulin secreation or insulin resistance.[4-6] The disease disturb blood sugar and the body metabolisim which is characterized by high blood sugar.[7] Nanotechnology is being used in different field, nanoparticles are now widely used as drugs to treat different disease and improved human health due to their antimicrobial of mortality action,[8] antibacterial, antiviral.[9,10] Application of engineered Ag and metal oxide NPS have evolved positively, influencing medicine. Infection is considered as afirst cause of mortality owing to wounds, after surgery.[11] P. *aeruginosa* have an important role in the infection after surgery from ancient time.[12] Nanotechnology puts together the capabilities, to manage the properties of materials b controlling their size and this has motivated carring out the researchers into numerous potential uses for nanomaterials.[13] The potent antibacterial properties of AgNPs, have been, started widely and provide hopful finding, for upcoming antibacterial and yeasts.[14-16] Silver is broad–spectrum antimicrobial inhibit growth on

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microorganisims.[17] The present study was conducted evaluate its antibacterial activity against P.*aeruginosa* contaminated diabetes induced by alloxane in mice.

MATERIALS AND METHODS

All procedures in this study were carried out in accordance with guidance of the animal Ethics Committee of faculty of pharmacy, All Mutansiriya Univrsity. Adult male swiss mice of seven week old (20–30)g were used in this study thirty mice were separated into six equal groups each five mice. To induce diabetes aloxan was given three groups induced diabetes by pre treatment with alloxan mg/kg day for three days given/ip. The blood taken from the tail for estimation of fasting blood glucose and plasma insulin level one group received distilled water (controls).Group 1 includeed non diabetic control, group 2 Diabetic, group 3 Diabetic+ o.1 Silver nanoparticles, group 4 Diabetic+ 0.5 silver nanoparticle, group 5 Diabetic + wound contaminated with *Pseudomonas aeruginosa*, group 6 Diabetic +infection +0.5 silver nanopaticle. Fasting blood glucose just before start in the experiment days for all animals.

Bacterial suspentionse

To prepare a bacterial suspention were culture in Mueller Hinton broth (Merk, Darmstadt, Germany) and in the log phase of growth, the suspention was centrifuged at 1000 g for 15 minutes. The supernatant was discarded and bacteria were diluted to 10 oss 8 CFU in sterile phosphate- buffered-saline > Ten microliter of the bacterial suspention (10 oss 6 CFU) were added to each wound bed after induction of full thickness skin defect, immediately.

Minimum inhibitory concentration (MIC) detection

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of an microorganisims after overnight incubation. The MIC values were determined based on amicro broth dilution method in 96 multi-well micro titer plates developed by Saker *et al* with slight modification[18] 50 micro liter of normal saline were added to each well of plate. Avolume of 100 micro liter of test material in added into the first row of the plate. Serial dilution were performed such that each well had total 100 microliter of the test material in serially descending concentrations. 10 microliter of resazurin indicator solution (prepared by dissolving a270 mg tablet in 40 mal of sterile distilled water) was added in each well. Finally 10 microliter of bacterial suspention concentration of 5multibly 10 oss6 CFU/mL was added to each well. Plate had acolum with streptomycin as positive control. The plate were prepared in triplicate and placed in an incubator at 37 c for 18–24 hours. Any colour change from purple to pink indicates growth of microbes. The highest dilution at which no nocolor change occurred was taken as the MIC value of drug.

Induction of diabetic

Adult male albino mice of seven weeks old (20 to 30g) were used in this study mice were divided into six group (n = 5) randomly kept under period of 12 hours light and 12 hours dark, specific pathogenic free conditions. The mice were kept on standard pellet diet and water ad libitum for two weeks to be acclimatized prior to the investigation. The base line of tail blood glucose level of each the animal was taken from normal control. glucose cheke, the blood glucose day using IP to establish diabetic mice. The animals with blood sugar levels>< mg/dc were considered diabetic daily monitory of blood glucose level. For the determination of blood glucose using with each of the two dose level of AgNps 0.1 and 0.5 mg/kg body weight either separately for 20 days. Diabetic develop gradually after 4 days.[19]

Animals the induced diabetic state was assed by daily monitory of blood glucose level. For the determination of blood glucose using glucoscheck, the blood glucose levels of these animals were measured at 30, 60, 120 miniutes intervals through tail tipping using aglucometer of the animals. The laboratory test of the animals, including fasting blood sugar (FBS).

Synthesis of Ag-Nps and kept in room temperature

AgNo3 (90ml, 1.0mM) was added drop wise under vigorous stirring by the use of magnetic stirring-cum-hotplate at ambient to 100 c silver nitrate solution and kept in room temperature. 1mm AgNo3 aqueous solution of silvernitrate was prepared for synthesis of silver nanoparticle. The reduction of metallic silvernanoparticle ions was monitored by measuring the UV-Vis spectra of the reaction mixture.

Characterization of synthesized silver Nanoparticle

Synthesis of silver nanoparticles was initially characterized by position of SPR band by measuring double beem UV-vis spectroscopy at different wavelengths from 360 to 700 nm. Crystal structure was characterized by XRD at 20 ranges from 10-to 90 shape and size were analysed by using SEM and TEM. Elemental composition was performed by EDAX. FTIR spectrum of silver nanoparticle was obtained on a SHIMADZU instrument with the sample as KBR pellet in the wave number region of 500-4, 000 cm^{-1}.

In *vivo* studies

Animals to domestic quality drinking water and food had adlibitum access. Twenty week old male swiss abino mice department animal house, college of pharmacy, all Mustansiriya university. Un reveserialy weighting between 26-30 gm were used for all study experiments. Diabetic induced and non diabetic induced control made mice were used for the impaired infection model healing.[21]

Infected wound model

The *Pseudomonas aeruginosa* obtained from the lab of microbiology in yarmuk hospital

For the experimental section, animals were divided into 6 groups carrier five animals. Group 1 with control. Group 2 diabetic. Group 3 Didiabetic and 0.1 AgNPs. Group 4 and 0.5 AGNPs. Group 5 Diabetic and infection. Group six diabetic +infection with P. *aeruginosa* +treatment with silver nanoparticle.

Anesthesia and wounds

All mice were anesthesized with 250 dose of ketamine – xylazine- saline cock tail (ratio 4:1:95. Consisting of ketamine (worden Holand) 100 mg kg and xylazre wodern Holand 5 and administrated 1/p.[19] Hairs of the mice Shaved and cleaned with 70 % ethanol and full thickness skin wound with 3mm in diameter created on the dorsal middle line of using sterile biopsy punch (Germany). The wound left open with any dressing material for the uration of the study.

The bacteria were grown in Mueller Hinton broth (Merck), Germany). When bacteria were in the lag face of growth, the suspention centrifuged at 1000 g for/min, the supernatant was discarded and the bacteria were diluted to 10 aus 8 CFU/mL add + each wound has immediately after wound surgery.[22]

Microbiological analysis

Swabs were taken from the wound during each dressing change on the day 3, 6, 9, 12, 15, 18, 21. The collected swabs were immediately sent to the laboratory for testing. In the qualitative count study, 2ml of normal saline was added to each of the samples. The vortex thouraly and 100 fold – serial dilution was performed. Eight hundered microliters of each sample dilution was replicates were carried out for each dilution and the agar plate were incubated at 37 c for 24 hours. The colonies were counted and results were tabulated.[20]

RESULTS

The characterization of synthesized nanoparticles by absorbtion spectra of Ag nanoparticles formed in the reaction media has absorbence maxima at 475nm. Apeak specific in the reaction media has absorbance maxima at 475 nm. Apeak specific for the synthesis of silver nanoparticle was obtained at 420-500 nm by UV-visible spectroscope.

It was found that silver nanoparticle could inhibit the bacterial growth in skin.

There was arapid reduction in wound size by by day in the nanoparticle –treated wound. Nanoparticle demonstrated areduction in bacterial growth in P.*aeruginosa contaminated* wounds.

This study results showed that all mice in alloxan induced diabetic group with significant decrease for insulin glucose level I/p adminstratin of silver nanoparticles AGNPs resulted in significance atidiabetic effect that is improved glucose tolerance, higher serum insulin (60 %) reduced blood glucose to (25%) revealed and sterility of the wound contaminated with *Pseudomonas aeruginosa*. The reduction in blood glucose level was more marked in normal in alloxan – diabetic mice. Treatment with single dose of AgNPs produced amild of antibacterial. Our result showed that AgNPs improved blood glucose level which therefore induced the possible role of AgNPs as cost –effective therapeutics medication in the treatment of diabetes. Table 1 showsthe effect of silver nanoparticle on induced diabetic. Table 2 shows the effect of topical application of AgNps 1.01, 0.05 on wound area. Table 3 shows the detection of bacteria in the wound fluid of mice in group treated with AgNps and treated without, Table 4 shows the effect of silver nanoparticle on induced diabetic contaminated with P.aeruginosa. Table 5 shows Bacterial average on wound area.

DISCUSSION

Nanomaterials with antibacterial activity that eleva te the effectiveness of antibacterial administration are called nanoantibiotics. The control of infection has been explored and demonstrated in *vivo* and *vitro*.

The present study has described the antibacterial properties as microscopic wound healing and diabetic by AgNPs. The AgNPs were evaluated as wound dressing material infected mice wound models.The present result showed that the topical application of silver nanopartinvicle is very effective in the bacterial load reduction in based on our finding the silvernanoparticle may reduce the bacterial load of wound infection so will accelerate the wounds healing. Result regard to its control of P. *aeruginosa* and its wound contraction

Table 1: The effect of silver nanoparticle on alloxan-induced diabetic mice.

Parameters	*Control*	*Diabetic*	*Diabetic +silver nanoparticle*
Blood glucose (mg/dL	65 70 75 85 100	120 320 150 200 250	60 55 40 30 20

Table 2: Effect of topical application of AgNps (0.01, 0.05 mg/kg) on wound are amm2

Control	*Day0*	*Day7*	*Day14*	*Day21*
Control	12.55	11.4+-0.14	6.33+-0.17	1.53+-0.75
AgNps (0.01)	12.54	9.65+-0.14	2.66+-0.17	0.0+-0.25
AgNps (0.05	12.54	9.96+-0.14	4.57+-0.18	0.10+-0.2

Percentage of wound contraction compared with day 0

Table 3: Detection of bacteria in the wound fluid of mice in groups treated with AgNPs without treated G G roups treated with AgNps Groups without treated.

Mice 1	*AgNps CFU*	*90.000 without treatment CFU*
Mice2	0	80.000
3	0	90.000
4	0	90.000
5	0	90.000

Table 4: The effect of silver nanoparticle on induced diabetic mice with contaminated with P. *aeruginosa*

	Before treatment	*After treatment*
Mice 1	18.6+-1	17+-1* 10^4
Mice 2	17.6+-2	16+-1 *10^2
Mice3	17.6+-3	15+-1 * 10^2
Mice 4	16.6+-2	13+- *10^1
Mice5	16.6+-1	Sterile no growth of bacteria

Table 5: Bacterial and average in wound area of experimental group post treatment

Nanoparticle	*Day*	
	4	10^4
	8	10^3
	12	10^2
control	4	10^4

effects on full thickness wound *in vivo* and *invitro*. Their experiment shows the positive effect of Silver nanoparticle for full thickness in an experimental animal models. The study agreement with Mohanty *et al*[24] who showed that AgNPs exhibit potent antibacterial activity. This study agreement with V Karthick *et al.*[25] who report the treated of AuNps shown significant reduction in blood glucose level on diabetic rat.This study agreement with Ravi Babu Birudu *et al.*[26] who report, silver nanoparticles have the antidiabetic activity. The silver nanoparticle had potential in *vivo* bactericidal effect against P. *aeruginosa*. And this result agreement with Massood *et al,*[27] who report that AgN ps invivo accelerating effect on the treatment of S.aureus infected skin wounds. In conclusion. The result of the present study, propose that treatment with AgNps adose of 0.01mg/kg of nanoparticle is safe effective in diabetic wound healing and kill this finding. P.*aeruginos so well accelerate the wound healing.* This result also agreement with Grciael *et al* (2018).[28] who report the E p/Agnps have good antidiabeticactivitity and there therefore could be used to prevent the development of diabetes. We conclude that AgNps antibacterial in applying nanotechnology in medicine for treatment of diabetes. further studies especially well designed clinical trials are required to confirm, the potential examination for the development of AgNPs as an antidiabetic factor in future.

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