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ORIGINAL ARTICLE

Comparative study of Antimicrobial Activities of Cu and Fe Nanoparticles against the Pathogenic Strain of *Acinetobacter baumannii*

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ABSTRACT
The use of metal nanoparticles can be effective to eliminate the bacterial infections, as an alternative to antibiotics. The aim of this study was to detect the antibacterial properties of 0.01, 0.05,0.08 and 1% nano-Cu and nano-Fe against Acinetobacter baumannii as a major and prevalent pathogenic bacterium. Acinetobacter baumannii was cultured in liquid and agar nutrient medium to evaluate the antibacterial effects of 0.01, 0.05, 0.08 and 1% of both nano-Cu and nano-Fe via the optical density (OD) and log CFU/ml measurements. Non-significant effect was seen for 0.01% of both nano-specimens. While, 0.05,0.08 and 1% of both nanoparticles showed considerably decreased bacterial number. A 4.1 and 1.7 times decrease in the OD value was found in the presence of 1 and 0.5% nano-Cu, respectively (P< 0.01). 1.2 and 3.5 times decreased OD was seen in the presence of 0.05,0.08 and 1% nano-Fe, respectively, as compared to control (P<0.01). In the second study, 6.3 log CFU/ml of Acinetobacter baumannii were present in the cultures treated with 1% nano-Cu and Fe at 4 ºC in water. Control Acinetobacter baumannii cells survived for 14 days while complete cell death was seen when 1% nano-Cu was applied for 15 hours as compared to 1% nano-Fe, which showed complete cell death after 17 hours. In the third study, Acinetobacter baumannii was grown in the agar medium with and without both nanoparticles and suppressed growth (4.5 and 5.6 times; P<0.01) was seen in the presence of 1% nano-Fe and -Cu, respectively. In spite of the fact that both nanoparticles showed bactericidal activity, nano-Cu has proven to be more efficient antibacterial agent as compared to nano-Fe.
***Keywords**: nanoparticle, macrodilution, bacteriostatic, bactericidal*

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INTRODUCTION

At present, the use of nano-structured materials is becoming more widespread and a major advantage over either organic or inorganic nanoparticles offers many possibilities of applications in the areas of physics, chemistry, pharmacy, surface coating agents, textile sizing, agriculture, biochemistry and so on [1]. It has been demonstrated that specially formulated metal oxide nanoparticles have good antibacterial activity and antimicrobial formulations comprising nanoparticles could be effective bactericidal materials [2]. Nano-materials are called "a wonder of modern medicine". It is stated that antibiotics kill perhaps a half dozen different disease-causing organisms but nano-materials can kill some 650 cells [3-6].

MATERIALS AND METHODS

Chemicals, growth media, and bacterial Strain *Acinetobacter baumannii* (ATCC 17978) was used for the present experiment. Nutrient Broth (BD234000; Becton Dickinson & Company, MD, USA) was used in growing and maintaining the bacterial cultures as per supplier's protocol. The chemicals such as; ascorbic acid, sodium citrate tribasic dehydrate, ammonium sulphate, ethanol and cetyltrimethyl ammonium bromide (CTAB) were purchased from Sigma and were of the highest purity available. These reagents were used as received without further purification. Cu and Fe nanoparticles, with the particle-size of 40

nm, were used throughout the experiment. The particles were suspended in sterile water and sonicated for 15 min before use (7).

Bacterial susceptibility to nanoparticles

To examine the susceptibility of *Acinetobacter baumannii* to nano-Cu and -Fe, three different estimation methods were used with three tiles repetition.

Bacterial growth in the presence of nano-Cu and Fe in liquid medium

In the first method, the bacteria were grown in nutrient broth (NB). To start the growth, 2 mL of the overnight-cultured *Acinetobacter baumannii* stock was added to 100 mL NB, containing 0.12% glucose with and without 0.01, 0.5 and 1% nano-Cu and Fe, separately. The bacteria were aerobically cultured at 37 ºC for 24 hours. Optical density (OD) measurements were taken at 600 nm to monitor the bacterial concentration.

Bacterial killing in the presence of nano-Cu and Fe in liquid medium

In the second method, the culture solution was centrifuged and the cells were washed and re-suspended in distilled water, reaching a final concentration of 6.3 *log* CFU/ml in each of the sample flasks and incubated at 4 ºC. The final concentration of the *Acinetobacter baumannii* suspensions was made in 100 ml distilled water. Different amounts of nano-Cu and -Fe (0.01, 0.5 and 1%) were then separately added to the bacterial suspensions to keep in contact with the bacterial cells and shaken at 4 oC for 48 hours. Optical density (OD) was measured to obtain the results. Aliquots of 0.1ml of the growth mixtures (water + bacterial cells + nanoparticles) were sampled every two hour. The number of resulting bacterial cells was noted after every two hours of incubation. Bacterial number was determined by measuring the optical density (OD) at 600 nm. The OD values were converted into the *Acinetobacter baumannii* concentration as *log* CFU/ml [8].

Bacterial growth in the presence of nano-Cu and Fe in agar medium

In the third method, the same bacteria strain was grown on a solid NB containing 0.12% glucose, 2% agar (control plates) alone or in the presence of 1% nano-Cu and -Fe. Bacterial cells were grown at 37ºC for 48 hours. Afterwards, the plates were visually estimated and bacterial colonies counted. The pictures were taken by an Olympus C2020Z digital camera. The data obtained in all tests were compared with the control. Student's *t*-test was used to evaluate the significance of experimental results (P<0.01).

RESULTS

Effect of nano-Cu and Fe on the growth of Acinetobacter baumannii liquid medium

In the first study, we investigated the effect of different concentrations of both nanoparticles in liquid culture of *Acinetobacter baumannii* . The optical density of the medium was investigated as the number of bacteria after contact with the nanoparticles. Fig. 1 shows the effect of different 0.01, 0.5 and 1% nano-Cu on the in growth and killing of *Acinetobacter baumannii* . As demonstrated by the figure, 0.01% nano-Cu did not have antibacterial efficiency on *Acinetobacter baumannii* but the concentrations of 0.5 and 1% nano-Cu inhibited the bacterial growth. Fig. 1 shows that 0.5% nano-Cu showed 1.7 times decrease the optical density of bacterial cultures (P<0.01) as compared to the control. While, in the presence of 1% nano-Cu, the optical density of *Acinetobacter baumannii* cultures decreased 4.1 times ascompared to thecontrol experiment. Fig. 2 shows the effect of different concentrations of nano-Fe on the growth of *Acinetobacter baumannii* .As demonstrated in this figure, 0.01% nano-Fe did not have antibacterial effect while, 0.5 and 1% nano-Fe was highly efficient in inhibiting the *Acinetobacter baumannii* growth as compared to control group. This figure shows that the presence of 0.5% nano-Fe caused a 1.2 times decrease in the optical density of bacterial cultures (P<0.05) as compared to control. As shownin Fig. 2, the presence of 1% nano-Cu caused a 3.5 times decrease in the optical density of *Acinetobacter baumannii* compared to the control group. Results of the Figs.1 and 2 show that nano-Cu have more efficient antibacterial property for *Acinetobacter baumannii* comparison with nano-Fe.

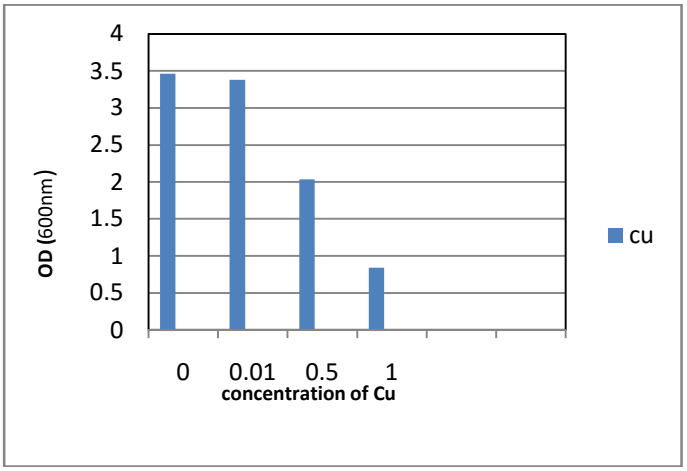

Fig. 1: *Acinetobacter baumannii* concentration dependence upon different concentrations of Cu in the culture medium.

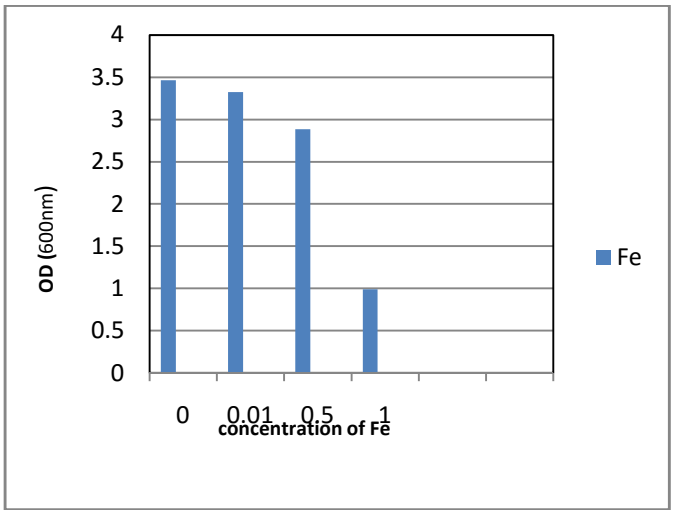

Fig. 2: *Acinetobacter baumannii* concentration dependence upon different concentrations of Fe in the culture medium.

Bactericidal effect of nano-Cu and Fe on Acinetobacter baumannii liquid medium

In the second study, estimation of the number of viable *Acinetobacter baumannii* cells in contact with 1% Cu and Fe was carried out in water at 4 ºC for different contact time intervals. Our result showed the reduction of *Acinetobacter baumannii* cells from 6.3 *log* CFU/ml to undetectable levels after 15 days (Data have not been shown). While, upon the addition of these nano-materials to the bacterial culture showed decreased survival rate within 2 days as compared to that of 15-day experiment for control group. Fig. 3 represents the number of viable *Acinetobacter baumannii* cells in contact with 1% nano-Cu and –Fe, separately, suspended in water at 4 ºC for different contact times. From the figure,it can be clearly observed that nano-Cu and -Fe exhibited different antibacterial properties. After the *Acinetobacter baumannii* were suspended in water along with Cu, the number of microbial cells reached zero after 22 hours. While, 1% Fe showed complete bacterial killing after 28 hours of their contact with 1% nano-Fe. These results demonstrate a stronger antibacterial effect of nano-Cu on the *Acinetobacter baumannii* as compared to nano-Fe. As compared to the control group where survival was seen upto a culture period of 15 day, the administration of nano-materials to the bacterial cultures killed the bacteria in less than 2 days. These results demonstrate that nano-Cu and Fe have a high antibacterial efficiency against *Acinetobacter baumannii* .

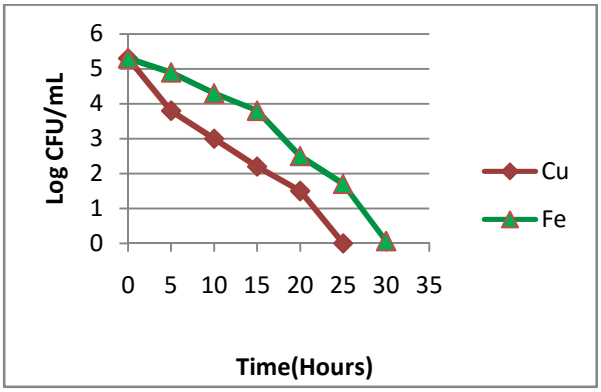

Fig. 3: Comparative killing kinetics of 1% Cu(▲) and Fe (●) on the A. *baumannii* cultures

Effect of nano-Cu and Fe on the Acinetobacter baumannii growth in agar medium

In the third investigation, *Acinetobacter baumannii* was grown on agar medium without (control) or with 1% nano-Cu and Fe, separately. Distinct bacterial colonies were observed in 105 times dilution. The visual estimation and bacterial colony counts were performed at this dilution. we can see smaller number of *Acinetobacter baumannii* colonies on the agar medium with nano-Fe B) and -Cu as compared to the control group. In the control plates, 970 ± 62 bacterial colonies were obtained while, in the experimental plates with 1% nano-Cu, 46 ± 15; and with 1% nano-Fe, 92 ± 21 bacterial colonies were seen. Thereby, nano-Cu suppresses the bacterial growth 21 times (P<0.05) in the agar medium as compared to that of 10.5 times decrease in the presence of nano-Fe.

DISCUSSION

The antibacterial activities of different concentrations of nano-Cu and Fe were investigated during the recent analysis. *Acinetobacter baumannii (ATCC 7881)* was used as the test organism during the experiments. Good growth inhibition results were observed when the bacterial cells were incubated with both kinds of nanoparticles during the liquid and solid cultures. The quantitative examination of bacterial activity was estimated by the survival ratio as calculated from the number of viable cells, which formed colonies on the nutrient agar plates [9]. Another study states the nano-Cu as a strong and effective bactericidal agent [10-13]. The present data demonstrate that a formulation made with the biologically stabilized Cu and Fe nanoparticles can be useful in the treatment of infectious diseases caused by *Acinetobacter baumannii*. A strong binding of nanoparticles to the outer membrane of *Acinetobacter baumannii* causes the inhibition of active transport, dehydrogenase and periplasmic enzyme activity and eventually the inhibition of RNA, DNA and protein synthesis, which finally leads to cell lysis as was seen for *Acinetobacter baumannii* during the present study [14]. It has been known that nano-materials exhibit strong inhibitory effects towards a broad spectrum of bacterial strains [15]. During the present study, different concentrations of nano-scale Cu and Fe were tested to find out the best concentration that can have the most effective antibacterial property against the *Acinetobacter baumannii* culture. Our data is in accordance with the previous studies, dealing with the antibacterial effects of nano-materials [16]. Several investigations have suggested the possible mechanisms involving the interaction of nano-materials with the biological macromolecules. It is believed that microorganisms carry a negative charge while metal oxides carry a positive charge. This creates an “electromagnetic” attraction between the microbe and treated surface. Once the contact is made, the microbe is oxidized and dead instantly. Generally, it is believed that nano-materials release ions, which react with the thiol groups (-SH) ofthe proteins present on the bacterial cell surface. Such proteins protrude through the bacterial cell membrane, allowing the transport of nutrients through the cell wall.Nano-materials inactivate the proteins, decreasing the membrane permeability and eventually causing the cellular death [17].

CONCLUSION

The present study reveals that the antibacterial effect of nano-Cu was stronger than that of nano-Fe, which can be because of the fact that the nanocomposition of Cu contained consequently more antibacterial active sites than Fe nano-particles.

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