Original Article

Comparison of antibacterial activities of cadmium oxide nanoparticles against *Pseudomonas Aeruginosa* and *Staphylococcus Aureus* bacteria

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Abstract

Background: Inorganic antibacterial factors have bacterial resistance and high thermal stability. Inorganic nanomaterials which have new structures with biological, chemical and physical properties have been made since their applications due to their nano size. In this study, the antibacterial effect of cadmium oxide nanoparticles on *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria was investigated.

Materials and Methods: The different concentrations (10 μg/ml, 15 μg/ml and 20 μg/ml) of cadmium oxide nanoparticles were prepared and their effects were studied against considered bacteria in both solid and liquid media.

Results: The results showed that there is a direct relationship between inhibitory effect and amount of consumer dose of nanoparticles. Furthermore, it was observed that antibacterial properties of cadmium oxide nanoparticles on activity and growth of *Staphylococcus aureus* was more effective than *Pseudomonas aeruginosa*.

Conclusion: This study showed that antibacterial effects of cadmium oxide nanoparticles on positive gram bacteria are stronger than negative gram bacteria and antibacterial effects of cdo nanoparticles against both bacteria, but *Staphylococcus aureus* bacteria were more sensitive to nanoparticles as compared to *Pseudomonas aeruginosa.*

Key Words: Cadmium oxide, environmental factors, nanoparticles, *Staphylococcus aureus*, *Pseudomonas aeruginosa*

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INTRODUCTION

Nano technology because of its features such as very small size and the ratio of surface area to mass is very useful, in addition used in biology and pharmacology, and it has some advantage in industries. furthermore, it causes to produce effective material, machines, systems with material control in nanometer length scale,[1-3] Numerous enzymes and toxins are produced by staphylococcus that cause survival of bacteria,[4] protein carbohydrate, lipid breakdown for providing material requirement, resistance to drug and pathogenic bacteria. Enterotoxins of this microbe are dispersed into the food or medium by bacteria cells. Their treatment features for wide range of microorganism are proved in urinary tract infection (UTI), respiratory tract infection (RTI), skin infaction, soft tissue infection, bacteremia; kinds of systemic infection

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especially in patients with severe burns, cancer, AIDS who have immunodeficiency can be created by pseudomonas aeruginosa. Pseudomonas inherently is resistant to many antibiotics, so is a resistance bacteria antibiotic which penetrates through the pores of outer wall of bacteria into pseudomonas; we expectthat nano materials with different methods of synthesis, have different anti-bacterial effects as well. so it is necessary to investigate its anti-bacterial effect.[5]

MATERIALS AND METHODS

Pseudomonas Aeruginosa and *Staphylococcus Aureus* are purchased from Shiraz University, and are proved by common methods of microbiology. Nutrient broth (NB) mediums (liquid) and Agar nutrient (solid) are imported by Merck Company in Germany. In sensitivity test of *Pseudomonas Aeruginosa* and *Staphylococcus Aureus* we used two different methods for estimation of correctness and confirmation of results.

Preparation and analysis method of cadmium oxide nano particle

In this paper for providing cadmium oxide nano particle, in one test solution sample first with 0.06 m Acetic a clad and 0.03 m cadmium sulfate by using 40 mg cetyl tri methyl ammonium bromide was prepared as surfactants in 1 liter water after two times of rectification second solution was prepared by 0.9 m aggregate gain and 25 ml ethanol 70% in 1 liter distilled water. After mixing of first and second solution the abstained precipitate was filtered by Whatman filter paper, then dried in hot air furnace in 80°C for 1 h. And then transferred to silica crucible and burned in 400°C for about 2 h, the obtained powder was washed for removing immurements 3-4 times by ethanol. Morphologic studies and investigation of synthesized cadmium oxide nano particle surface are done by visual spectrometer double beam ultraviolet model Tu-1901, X-ray diffraction model D/max – RA, radiation cuk and electrical microscope model JEM –200cx.

Bacteria cultivation and effect of cadmium oxide nano particle in solid medium

Sterile swab were immersed in liquid medium (NB) of bacteria cultivation and were stained to bacteria, then they cultivate in some plate to stain to the bacteria completely. After that the mentioned solution selected from average and constant density 15 μg/ml for standardization among three densities of 10, 15, 20 μg/ml and the powder poured in three wells separately and the solution was poured in wells that here in distilled water. Then plates were placed in incubator at 37°C for 24 h and after they were counted to determine the effect of bacterial colonies nano particle.

Preparation of TSB (trypticase soy Broth) medium, Bacteria cultivation and investigation of cadmium oxide nano particle in liquid medium

Bacteria were cultivated in four separate tubes and from each four categories one tube is considered as control group. Then in remaining tubes, three kinds of density of cadmium oxide were added, and the tubes were sealed with cotton and shaked with Aerobic at 37°C for 24 h in incubator. Then optical density is used for determining of bacteria density.

Bacteria culture and exploring the effect of cadmium oxide nanoparticles in solid media

First, we created well using loop in four specific points in the solid media, three of which were for three selected concentrations of cadmium oxide nanoparticles and the fourth one that was created in the middle of the plate was for the control group. Then we inserted the sterile swab in NB fluid bacteria media that had been prepared before and *Staphylococcus Aureus* and *Pseudomonas Aeruginosa* had grown in it (24-hour culture) and we impregnated it with bacteria and then we rubbed it to the plates containing solid media in three directions so that the plate surface is fully impregnated with bacteria. Then we chose the nanoparticles solutions that were prepared in fixed mean concentration of 15 μg/ml for standardization among 10 μg/ml, 15 μg/ml and 20 μg/ml and poured it into a specific well and we poured the control solution, which is water here, in the middle wall. (The method for obtaining different concentrations i.e. 10 μg/ml, 15 μg/ml and 20 μg/ml: We dissolved 10 Mg of the nanoparticle in 100 ml water for obtaining 10 μg/ml concentration of the nanoparticle and the intended concentration was obtained. And for obtaining 15 μg/ml and 20 μg/ml concentrations of the nanoparticle, we dissolved 15 and 20 mg of the nanoparticles separately in 100 ml water each). Then we placed the plates in incubator at 37°C for 24 h and then we explored the effect of nanoparticles on bacteria growth and then the bacteria colonies were counted. The pictures have been taken by Olympus C2020Z digital camera [Figure 1].

Data analysis

The obtained results are compared by control group and SPSS software and Dunnett's test is used for determining the significant results of tests and their estimation.

RESULTS

X-ray diffraction for cadmium oxide nanoparticles

X-ray diffraction is created by a set of atoms which is due to the reinforcement of scattered ray in special

space direction. After x-ray hits the material electrons it makes them oscillate and these electrons result in x-ray radiation around them with the frequency of the original ray. Figure 2 shows x-ray diffraction for cadmium oxide in which the diffraction is absorbed at 2 θ. Significant peaks are employed for estimating the size of the sample particles using Scherrer equation $D = K\lambda/(\beta\cos\theta)$ where K is constant and is equal to 0.9, λ is wavelength and A°) (Cu Kα) 5418/1=λ);, β is all the width of line's half maximum and θ is diffraction angle. The particle size is estimated using peak severity ratio (100). For cadmium nanoparticles, the particle size is found to be 30 nm and increase in the sharpness of the peaks of X-ray diffraction indicates that they are crystal in nature. The reflections that are clearly seen are closely compatible with cadmium oxide reference models.

Absorption spectra UV- Vis cadmium oxide nano particle

This spectrometry is related to transition between electronic levels such transitions is implemented between hybrid orbitals or unshared pair of electrons with non- hybrid orbitals. As a result, the wavelength absorption peaks can be associated with a variety links in studied species, through visible absorption spectra ultraviolet of cadmium oxide nano particle is shown in Figure 3. However, spectrometer wavelength is limited to light source, but the absorption bands of nano particle showed a blue shift that is due to the amount of limitation in sample, in comparison with mass of cadmium oxide. This optical phenomena show that these nano particles show amount of quantum effect.[6] Here nano particle constitute is related to surfactant and organic solvent, because surfactant of cetyl tri methyl Ammonium Bromide (CTAB) helps to bind the synthesized nano particle.[7] So as a result of this, stabilizing effect of particle and the formation and growth of the core particles are based on high degree of uniformity acetic acid and ethanol solvent helps in diffusion of particles uniformly in limited size and prevent from particle concentration.[8]

Investigation of electronic microscope

In Figure 4, it can be observed the image of synthesized cadmium oxide nano particle. This image is obtained by TEM microscope with magnification of 1300 times that show synthesize nano particle diameter is about 30 nm.

Exploring the inhibitory effect of cadmium oxide nanoparticles on *Staphylococcus Aureus* and *Pseudomonas Aeruginosa* in solid media

Table 1 shows the antibacterial effects of cadmium oxide nanoparticles on *Staphylococcus Aureus* and *Pseudomonas Aeruginosa* in solid media. Different concentrations of nanoparticles were used namely

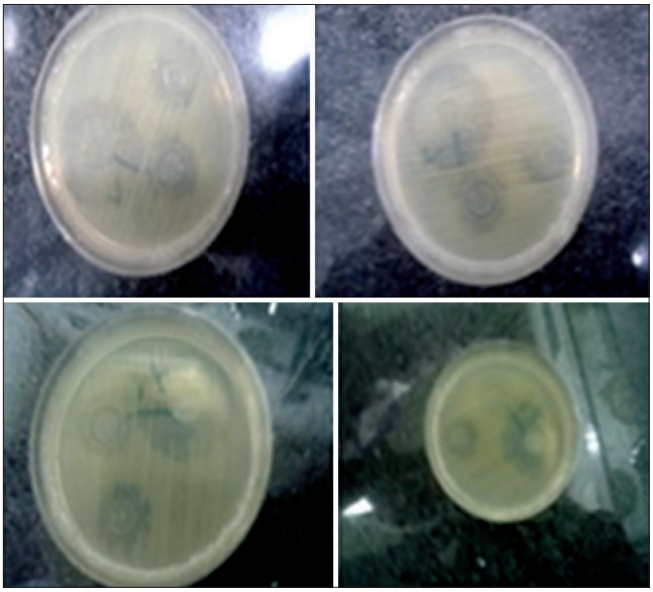

Figure 1: Solid media for the studied bacteria taken with digital camera

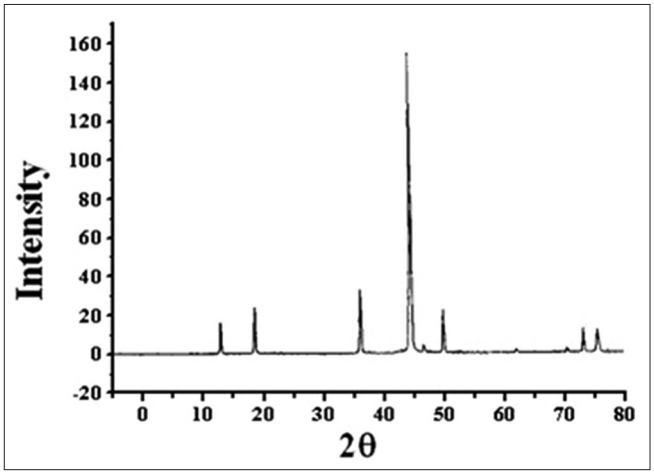

Figure 2: X-ray diffraction samples of cadmium oxide nanoparticles

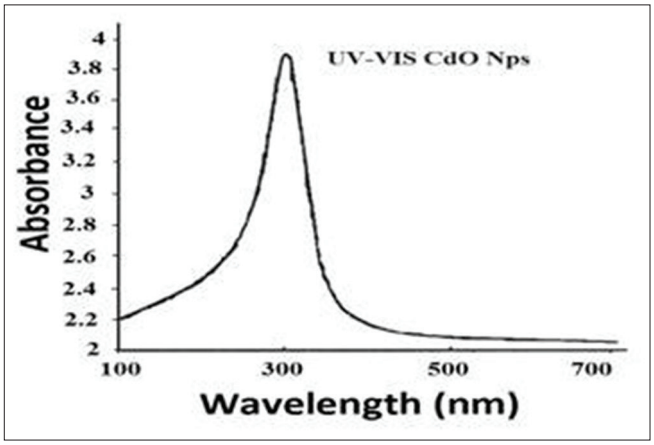

Figure 3: UV absorbtion spectru for cdo nano particle

10 μg/ml, 15 μg/ml and 20 μg/ml. In fact the growth and the activities of the bacteria against these concentrations have been studied. As it can be seen, the lack of growth shade that *Staphylococcus Aureus* has formed against cadmium oxide nanoparticles indicating that the bacteria growth has been highly stopped and there is a direct relationship between the inhibitory effect and the consumed dose of the nanoparticles and it can be used as a material with antibacterial properties. As it is seen, the diameter of the lack of growth shade that *Pseudomonas Aeruginosa* has formed against cadmium oxide nanoparticles is smaller compared with that of the *Staphylococcus Aureus* which indicates the higher resistance of *Pseudomonas Aeruginosa* to cadmium oxide nanoparticles.

As Table 1 shows with the increase of cadmium oxide nanoparticles, the diameter of lack of growth shade is increased too.

Investigation of inhibitory effects of cadmium oxide on *Pseudomonas Aeruginosa* and *Staphylococcus Aureus* in liquid medium

In investigation of cadmium oxide effect on *Pseudomonas Aeruginosa* and *Staphylococcus Aureus* in liquid medium we get that cadmium oxide with 20 μg/ml density has most inhibitory effect on bacteria and the effect of cadmium oxide nano particle on *Staphylococcus Aureus* bacteria than *Pseudomonas Aeruginosa* is more, because of the optical absorption in this bacteria in comparison to control group decreased more. For more accurate analysis one can see comparative diagram in Figure 5.

In this study the effect of different densities of cadmium oxide on number of *Pseudomonas Aeruginosa* and *Staphylococcus Aureus* bacteria is done. As you can see in diagram 6, the inhibitoriest effect is on high density of nano particle and control group with 0 mg/kg has not received nano particle and has highest growth [Figure 6].

In *Staphylococcus Aureus*, the number of cell with consumption of nano density has direct relation and from regression coefficient outcome we conclude that this relation is negative. It means whatever concentration rise we observe low number of cell on $P < 0.05$ and show there is significant relation. In *Staphylococcus Aureus*, the number of cell to consumption concentration has direct relation and we conclude that this relation is negative, it means whatever concentration rise we observe low number of cell and $P < 0.05$ and this is significant relation.

Investigation of temperature effect on *Staphylococcus Aureus* and *Pseudomonas Aeruginosa* bacteria in liquid medium

For growth of *Staphylococcus Aureus* bacteria optimum temperature is between 35 and 37°C and for *Pseudomonas Aeruginosa* bacteria is 30-37°C. This result is consistent to physiology of this bacterium. Figures 7 and 8 show the results of temperature on mentioned bacteria, in the presence of cadmium oxide. These results are consistent to inherent

Table 1: The results of measuring the diameter of lack of growth shade for the effect of different concentrations of cadmium oxide nanoparticles on the studied bacteria

Cadmium oxide concentration / Bacteria	Control	10 mg/ml	15 mg/ml	20 mg/ml
Staphylococcus Aureus	0.0 mm	10±2 mm	14±2 mm	19±2 mm
Pseudomonas Aeruginosa	0.0 mm	5±2 mm	10±2 mm	16±2 mm

As table 1 shows with the increase of cadmium oxide nanoparticles, the diameter of lack of growth shade is increased too

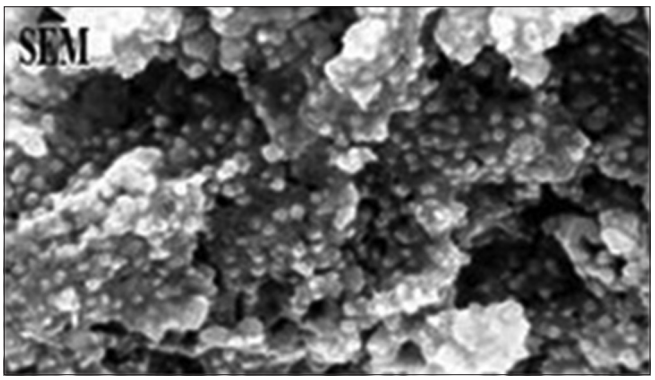

Figure 4: TEM image from synthesized cadmium oxide nano particle by magnifying 13000 times

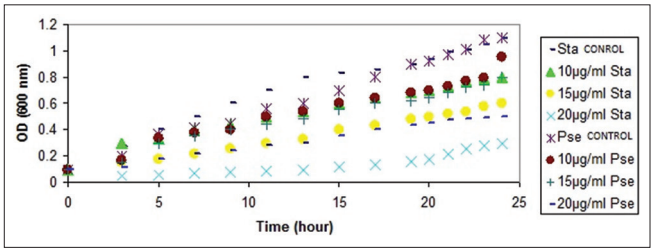

Figure 5: Comparison of different density of cadmium oxide on effect *Pseudomonas Aeruginosa* and *Staphylococcus Aureus* bacteria

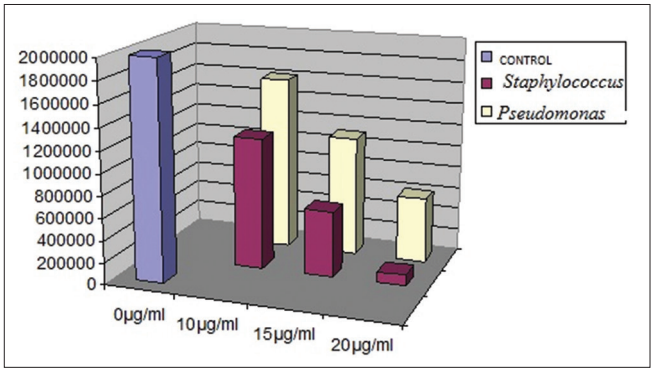

Figure 6: Comparison of different density of cadmium oxide on number of *Pseudomonas Aeruginosa* and *Staphylococcus Aureus* bacteria

physiology of this bacterium against temperature. As it shows, because of double effect of nano particle and temperature change, there will be significant decrease in number of cells.

Optimization of antibacterial effect of cadmium oxide nano particle in different times

In final study, live cells of sand *Pseudomonas Aeruginosa* bacteria were on the other hand maximum concentration of cadmium oxide nano particle (20 μg/ml) in 37°C in water. The results show that in control group, decrease of suspension concentration of *Pseudomonas Aeruginosa* and *Staphylococcus Aureus* from 6.3 log CFu/ml to undetectable concentrations review is achieved after 14 days but with addition of nano particle to mediums the viability of bacteria is decreased from 14 days to 2 days. As it is shown in Figure 9, *Staphylococcus Aureus* bacteria is more sensitive than cadmium oxide and its activity is more decreased than to *Pseudomonas Aeruginosa* bacteria.

Statistical analysis about *Staphylococcus Aureus* with rise of concentration and time cause to decrease OD and there is significant relation between OD and time and consumption concentration of nano particle. Also in two ways ANOVA the same premise of the OD is rejected, because ($P < 0.05$) and statistical analysis about *Pseudomonas Aeruginosa* with increase of time and concentration, cause to decrease of OD and there is not significant relation between OD and time and consumption concentration of nano particle because of ($P > 0.05$) two way ANOVA is not rejected.

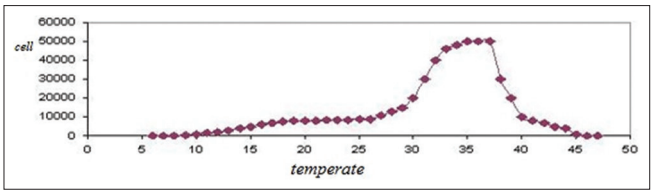

Figure 7: Effect of temperature on *Staphylococcus Aureus* bacteria in presence of cadmium oxide nano particle.

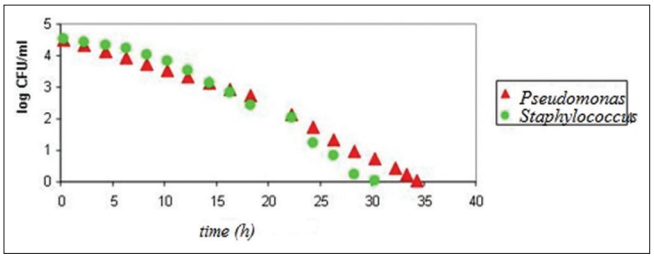

Figure 9: Comparison of maximum concentration (20 μg/ml) effect of cadmium oxide on *Pseudomonas Aeruginosa* and *Staphylococcus Aureus* viability

The comparison of the inhibitory effect of cadmium oxide nanoparticles on the number of the wild and modified *Staphylococcus Aureus* and *Pseudomonas Aeruginosa*

In another part of the study, the effect of cadmium nanoparticles on the number of the wild and modified *Staphylococcus Aureus* and *Pseudomonas Aeruginosa* was studied and the results indicated that the wild species (samples collected from the treatment section of hospital) showed more resistant to cadmium oxide nanoparticles. But overall, these results confirmed the antibacterial properties of cadmium oxide. The details are presented in Figure 10. In this chart, the letter C represents the control group and the letter W represents the wild species and Sta is abbreviation of *Staphylococcus Aureus* and Pse is the abbreviation of *Pseudomonas Aeruginosa.*

Investigation of pH effect on *Staphylococcus Aureus* and *Pseudomonas Aeruginosa* bacteria in liquid medium

In this study the effect of pH on *Staphylococcus Aureus* and *Pseudomonas Aeruginosa* in the presence of cadmium oxide nanoparticles was explored and the results are shown in Figure 11. Due to the double effect of both nanoparticles and pH changes, a significant reduction in the cell numbers happened.

In the next study, the effect of temperature on *Pseudomonas Aeruginosa* in the presence of cadmium oxide nanoparticles was explored and the results are shown in Figure 12. Due to the double effect of both

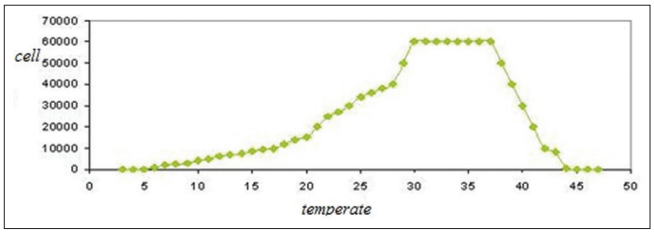

Figure 8: Effect of temperature on *Pseudomonas Aeruginosa* bacteria in presence of cadmium oxide nano particle.

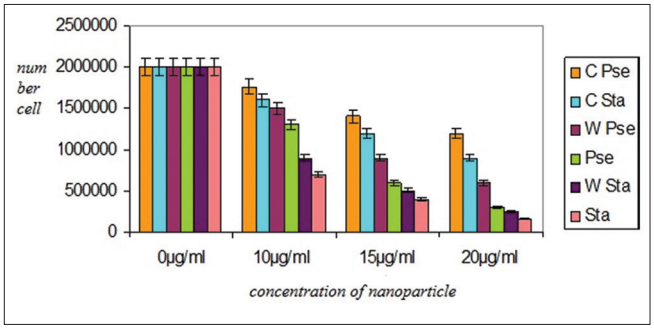

Figure 10: Comparison of the inhibitory effect of cadmium oxide nanoparticles on the number of the wild and modified *Staphylococcus Aureus* and *Pseudomonas Aeruginosa*

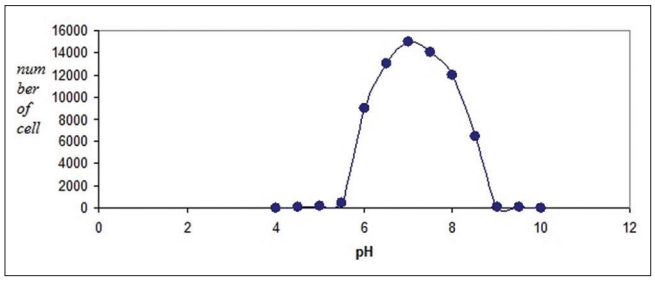

Figure 11: The effect of pH on *Staphylococcus Aureus* in the presence of cadmium oxide nanoparticles

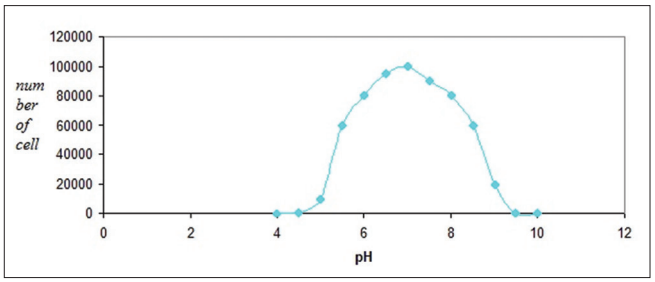

Figure 12: The effect of temperature on *Pseudomonas Aeruginosa* in the presence of cadmium oxide nanoparticles

nanoparticles and pH changes, a significant reduction in the cell numbers happened.

DISCUSSION

Nanoparticles have many applications in medicine as antibiotics only eliminate a very small number of pathogens while the nanoparticles eliminate about 650 types of pathogens.[3-5] In this study, two bacteria that are resistant to antibiotics were used and cadmium oxide nanoparticles at 20 μg/ml showed antibacterial effects on them and these nanoparticles can be used for eliminating the resistant bacteria. And in a study that was conducted by Buzby *et al*., the antibacterial effects of silver nanoparticles were explored and the researcher used 10-15 nm nanoparticles and showed that the increase of nanoparticle effects is dependent on the consumed doses.[9] In this study too, it was found out that with the increase of the number of cadmium oxide nanoparticles, the antimicrobial property is increased and the growth speed of the bacteria is decreased. In another study that was conducted by Sundrarajan, the antibacterial effects of Magnesium oxide nanoparticles on gram-positive *Staphylococcus Aureus* and gram-negative *Escherichia coli* was explored and it was indicated that the diameter of lack of growth shade for gram-positive bacteria was bigger than that of the gram-negative bacteria.[10] Also, in the present study the antimicrobial effect of cadmium oxide nanoparticles is stronger on gram-positive bacteria. In another study, the researchers explored the antibacterial effects of Cerium oxide (CeO_2) on *Staphylococcus Aureus*. The size of the nanoparticles used in them was 37.6 nm and the results indicated that the antibacterial properties of Cerium oxide are dependent on the consumed dose and the inhibition of these nanoparticles proved the antibacterial effects of the nanoparticles.[11] Ayaa *et al.,* (2009) were able to inhibit Methicillin-Resistant *Staphylococcus Aureus* using silver nanoparticles. Using Well Diffusion Agar, they proved the antibacterial effect of silver nanoparticles. Then they were able to determine minimum inhibitory concentration for it using microdilution method.[12] In a study, Rafie *et al.,* (2010) in Egypt were able to control *Escherichia coli* and *Staphylococcus Aureus* in cotton using nano silver.[13] Barzegari *et al.,* (2010) studies the effect of Titanium oxide on *Staphylococcus* and concluded that this nanoparticle has a good antibacterial effect on this gram-positive bacterium[14] and the present study has found this antibacterial effect too. Some studies were conducted on the toxicity of Cro nanoparticles on gram-positive bacteria of human immune cells and gram-negative bacteria such as Shigella which indicate the toxin nature of Cro nanoparticle for different microbial systems and human T lymphocytes.[15] In 2011 researchers studied the effect of CoFe2O4, CrO nanoparticles on *Staphylococcus* and the results indicated that CrO has more antibacterial power than $CoFe_2O_4$ against *Staphylococcus Aureus* and that overall both nanoparticles have antibacterial effect but CrO has a better performance[16] and we confirmed the antibacterial effect of cadmium oxide nanoparticle in this study. At first, nanoparticles result in peroxidation of polycyclic phospholipid compounds of the lipid membrane of *Staphylococcus Aureus* and *Pseudomonas Aeruginosa* and consequently the integrity of cell membrane is reduced and the basic activities in cell structure including respiratory activities are eliminated and cell death becomes inevitable. Considering the previous studies, it seems that lipid compounds of *Pseudomonas Aeruginosa* cell membrane in the presence of nanoparticles are more resistant than lipid compounds of *Staphylococcus Aureus* cell membrane. An accurate mechanism by which nanoparticles inhibit microbial growth is not fully understood. Generally regarding possible mechanisms of nanomaterial's possible reaction with biological macromolecules it is believed that nanomaterial release ions that reacts with thiol group (SH-) of the existing proteins on bacteria cell. Such proteins have outward bulge from bacteria cell membrane and result in the transfer of nutrition from cell wall.[17] Nanomaterials deactivate these proteins, reduce membrane permeability and ultimately result in cell death. In the present study, it is observed that with the increase of cadmium oxide nanoparticles

concentration, the growth is reduced. This can be interpreted in this way that cell damage is done with a higher speed and the antimicrobial effect is stronger. Nanomaterials may be able to reduce microbial adhesion and biofilm formation. We hope that in future, using these nanoparticles in the form of combinations or pure, pathogenic microbes in special cases such as epidemics or in the case of emerging or reemerging bacteria or microbe spread are easily dealt with and dangerous conditions are resolved.[18] In another study that was conducted by Sundrarajan *et al.*, the antibacterial properties of Magnesium nanoparticles on gram-positive *Staphylococcus Aureus* and gram-negative *Escherichia coli* was explored and the results showed that the diameter of lack of growth shade for gram-positive bacteria was bigger than that of the gram-negative bacteria.[6] Also, in the present study the antimicrobial effect of cadmium oxide nanoparticles is stronger on gram-positive bacteria. Another finding of the study is that if the temperature for drying and preparing nanoparticles is higher, their antibacterial properties are decreased. In another study that was conducted by Masound Negahdai *et al.*, the antibacterial properties of Cerium oxide (CeO_2) nanoparticles on *Staphylococcus Aureus*. The size of the nanoparticles used by them was 37.6 nm and their results indicated that the antibacterial properties of Cerium oxide are dependent on the consumed dose and the inhibition of these nanoparticles proved the antibacterial effects of the nanoparticles.[19] Recently in a review study, Hajipour *et al.*, explored the role and importance of the antibacterial properties of nanoparticles[20] and their results are consistent with those of the present study.

CONCLUSION

In this study with the increase of cadmium oxide concentration, anti-microbial features increase and bacteria growth speed is decreased, that is consistent to other research about nano particle effect on microorganism. One can say that in presence of nano particle cell damage occurs more rapidly.

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