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Study of the effect of anti-bacterial nano-particle Fe_2NiO_4 on the bacteria Escherichia coli and Klebsiella pneumoniae causing urinary infection resistant to some anti-biotics

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ABSTRACT: Background and Purpose: Using metal oxide nano-particles to fight back bacterial infection can be considered an alternative method of anti-biotics. In this article, anti-bacterial properties of nano-particles Fe_2NiO_4 to fight back Escherichia coli and Klebsiella pneumoniae were studied as one of the important and common bacteria in urinary infection.
Material and Methods: Well diffusion method was used to measure sensitivity of the bacterial mentioned to nano-particle Fe_2NiO_4. All experiments were performed in triplicate and soft wares SPSS/16 and statistical model MANOVA were used for statistical analysis of the data.
Results: The best concentration of nano-particle Fe_2NiO_4 was 125 mg/ml. Moreover, the highest value of inhibition areola in Klebsiella pneumoniae and Escherichia coli was an average of 94/20 and 22 mm respectively.
Conclusion: Findings showed that nano-particle Fe_2NiO_4 had more power to kill the Bactria against Klebsiella pneumoniae and Escherichia coli.

***Keywords*:** Nano-particle Fe2NiO4, Urinary Infection, Antibiotic Resistance

INTRODUCTION

The belief that technology is another era of science and it is a mixture of biology, chemistry, medicine and physics were accepted by scientists. Studies have shown that the smaller the particle size, they represent different new characteristics and activities. Such properties resulted in the widespread use of nano materials in such a way that their application are recognized in all dimensions of life like: electrical systems, fighting germs, diagnosing and curing diseases.

Moreover, nano-particles were discussed in two parts: metal and non-metal. Metal nano-particles have been used in bactericides and pesticides for a long time. Some nano-particles are considered as a novel method in development of the modern pharmaceutics science having many applications in biological and pharmaceutical sciences. For instance, they are capable of destroying 650 cancer cells in less than 4 hours. Nan--particles have shown the lowest level of toxicity in ecosystem and life cycle. Therefore, using such materials can be a good choice to fight pathogenic microbes. Previous studies showed that nano-particles like ZN, TI, AG and CR have a much higher bactericidal properties.

Escherichia coli and Klebsiella pneumoniae belong to negative warm bacteria having a cell wall equal to 7-8 nano-meter covered with a Lipopolysaccharide layer of no strength. These bacteria cause diarrhea and disorder in digestive and reproduction system. There were many studies in the experimental level to discover alternative materials for antibiotics after emergence and increase in bacterial resistance. Development of antibiotic-resistant bacteria is one of the problems that doctors face. Therefore, number of effective and available antibiotics has decreased to cure such infections. As a result, it is necessary to find new ways of therapy and making new drugs.
Metal oxide nano-particles show a different antibacterial property according to the ratio of surface to volume. Positive warm bacteria show more resistance to metal nano-particles than negative warm bacteria which can be attributed to structure of its molecule wall. Many have been done possible reactions between nano-particles and macro-molecules of living organisms. Difference between micro-organism's negative charge and nano-particles' positive charge acts as an electromagnetic absorber between microbe and nano-particle connecting nano-particle to the surface of the cell and this can kill the cell. Finally, a large number of such connections lead to oxidization and quick death of microbes' surface molecules. It is estimated that released ions from nano-materials react with Thiol groups (SH) of surface proteins in bacteria cells. Some of these bacterial cell membrane proteins are responsible for transfer of minerals from the wall surface. Therefore, nano-materials make the membrane inactive and impermeable through affecting these proteins.

Inactivation of membrane permeability will cause cell death at the end. Moreover, nano-particles delay sticking of bacteria cell and biofilm formation which results in disability of some bacterial to become fixed and proliferated. Anti-bacterial changes preventing pathogenic bacteria growth are considered a suitable purpose. Factors which cause infections can be diverse. Colonization, bacteria cell growth and formation of compressed microbial biofilm matrices make bacterial resistant to the host's immune system. Therefore, nano-particles prevent from forming these microbial defensive factors against host's immune system. Nano-particles basically formed from metal ions have a wide cell killing nature against bacteria, fungus and virus. Nano -materials especially metal nano-particles deactivate enzymes and micro-organisms DNAs through making electron balance between electron donor groups like: thiol, carboxyl, amide, imidazole, indole and hydroxyl due to surface charge and surface to volume ratio.
This study aimed to investigate the effect of anti-bacterial nano-particle Fe_2NiO_4 on Escherichia coli and Klebsiella pneumoniae MDR responsible for urinary tract infections using Well Diffusion Method in order to use nano-particles Fe_2NiO_4 in pharmaceutical industry instead of antibiotics to cure urinary infection against so-called bacteria.

Material and Methods

Material and method of studying bacteria: We used 24 isolated bacteria of Escherichia coli and Klebsiella pneumoniae each having 12 samples. Samples were gathered from many medical laboratories in Isfahan.then, they were diagnosed with biochemical tests and resistant samples to some antibiotics were selected. We used nutrient agar medium to grow bacteria, trypticase soy broth to enrich bacteria and Mueller-Hinton agar medium to determine sensitivity to nano-particles.
Providing Nanoparticles: The Nano-particle under investigation was bought from Sigma Company in US. We took TEM picture to study nano-particle sizing by Phillips electron microscope model H600 to confirm nano-particle size (figure 1). Nano-particle solution would be put on shakers to distribute nano-particle in agar medium before use. Then, the medium was took place in the shaker incubator. Moreover, amount of nano-particle inoculated was not such that causes precipitation or even distributed.

Research Method

At first, 0.5 McFarland suspension of the bacteria tested was prepared in sterile normal saline and it was cultured by sterile swab on Mueller-Hinton agar medium in three dimensions. Then, trenches 6 mm in diameter were created on agar medium. We picked up 1ml of stock solution of nanoparticles Fe2NiO4 with a concentration equal to 1000 mg/ml to determine anti-microbial effect of nano-particle Fe_2NiO_4 and solved in 1 ml of deionized distilled water solvent to yield concentration equal to 500 mg/ml. This dilution continued to a concentration of 5/62. Then, we put 80 micro liter of each of these dilutions in every trench and incubated at a temperature of 37 °C for 24 hours. The inhibition areola was measured after this period.

Results

The TEM picture of this nano-particle was taken to study nano-particle diameter and its surface to volume ratio as follows. It was shown that nano-particle diameter is 60 nanometer.

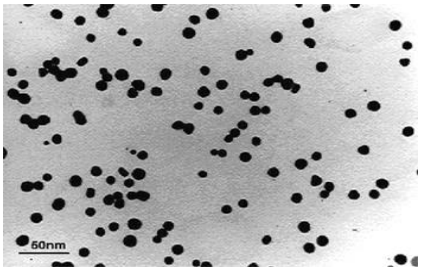

Figure 1: TEM picture of nano-particle Fe_2NiO_4

Lethal concentration of this nano-particle on Escherichia coli and Klebsiella pneumoniae bacteria was shown in graph1. There was sign of inhibitory effect in so-called bacteria in all concentrations made from nano-particle solution Fe_2NiO_4 according to graph 1. Moreover, there was a more inhibition areola in both bacterias at a concentration of 125 milligrams per ml nano-particles.

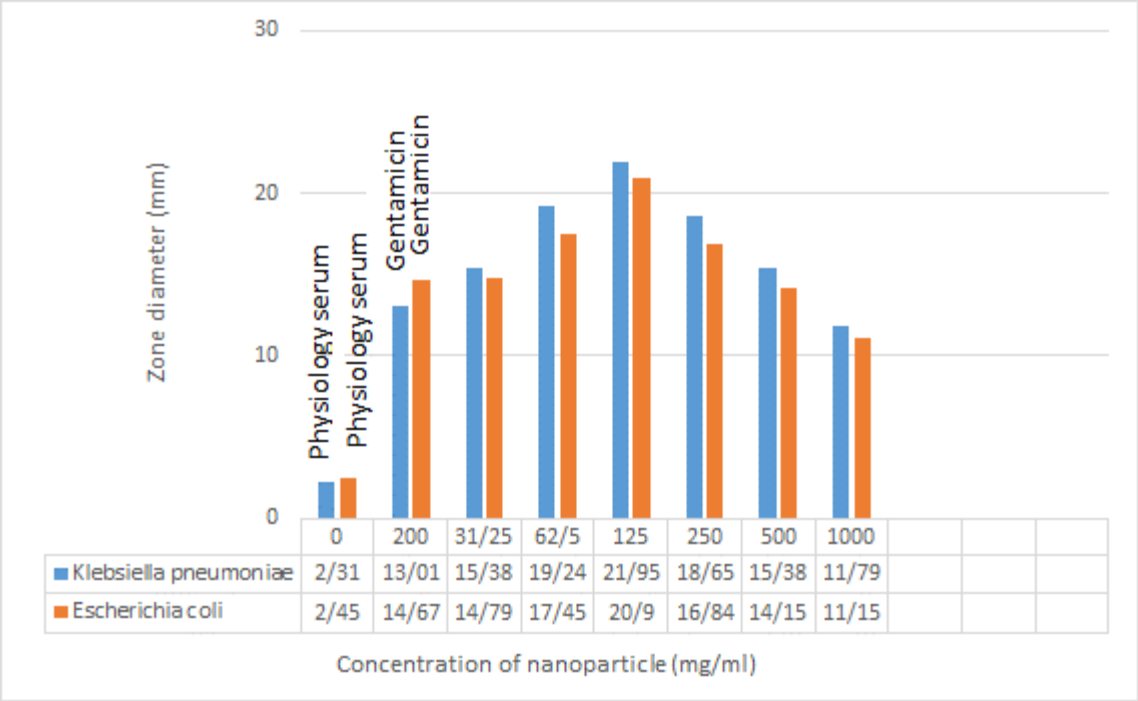

Figure 1: Inhibition areola diameter average in Escherichia coli and Klebsiella pneumoniae bacteria in different concentrations of nano-particles Fe_2NiO_4 using well diffusion method

Discussion

In this article, antibacterial activities of different concentrations of nano-particle Fe_2NiO_4 on Escherichia coli and Klebsiella pneumoniae bacteria were studied. It is hoped that these materials be used for the treatment of urinary infections in the future. This nano-particle had a suitable performance with different proportions regarding prevention of bacteria growth.

It has been approved that damage for humans has no relationship with production volume and risk of exposure to nano-particles but it is related to reactivity and power to affect biological systems such as the immune system. Therefore, engineered nano-particles at lower values having a strong effect on immune system like: cobalt and nikle nano-particle can have significant danger to human health. Nano-particles consisting elements like: cobalt, iron and nikle with magnetic properties are called magnetic nano-particles. Nano-particle Fe_2NiO_4 used in this article had megnetic properties.

Magnetic nano-particles are used in different medical conditions. Nano-particle Fe_2NiO_4 was used for treatment of urinary infections by Escherichia coli and Klebsiella pneumoniae bacteria in experimental conditions. Nickel

oxide is a compound with very low solubility and high biologic resistance power in lungs. Our nano-particle had a low solubility either and most iron particles would be deposited. The nano-pots were on shakers in order for nickel to be solved before use.

Iron oxide crystals with antibody molecules and peptides were used to bind to tumor cells. These nano-particles can target and connect to tumor anti genes or vascular problems with specific binding properties. Results of well diffusion method showed that nano-particle Fe_2NiO_4 has a strong bactericide property. Iron oxide crystals of nano-particle Fe_2NiO_4 could connect to bacteria and showed anti-bacteria activity. It has been shown that strong binding of nano-particles to the outer bacteria membrane can prevent from dehydrogenate enzyme function. Preventing from bacterial periplasmic enzyme activity and thus preventing the activity and function of RNA, DNA and protein synthesis were also seen. In general, this prevention of activities will lead to cell lysate. Findings of this stuy also confirm these results.

Furthermore, it has been proved that cobalt and iron (compared to other metal oxide nano-particles like silver) have a better adaption bio-effects and represent better influencing power. Therefore, they are a suitable option factor for practicality of these nano-particles to combat and prevent from these pathogenic factor. Nano-particle Fe_2NiO_4 was used in this study to cause death of Escherichia coli and Klebsiella pneumoniae bacteria MDR and prevented from overconsumption of antibiotic drugs by the patients no to change into bacteria sensitive to MDR.

Previous studies showed that chrome and iron oxide nano-particle have influenced growth of most warm positive and negative pathogenic bacteria like: Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli and prevents from bacteria growth. Level of preventive concentration from bacteria growth varied according to resistance of bacteria type studied. For instance, studies of Imani et al. (1388) showed that using nano-particles can prevent from growth of pathogenic bacteria like Shigella, Escherichia coli and Staphylococcus aureus being main causes of food contamination and seasonal bloody diarrhea. However, this study aimed to investigate the effect of nano-particle Fe_2NiO_4 on two types of bacteria Escherichia coli and Klebsiella pneumoniae and its anti-bacteria property has been proved. It is expected that nano-particles be used for treatment of urinary infection by Escherichia coli and Klebsiella pneumoniae bacteria.

Conclusion

Different concentrations of nano-particles Fe_2NiO_4 were studied to find that concentration of nano having the highest property of bactericidal. Finally, it was shown that antibacterial property of nano-particle Fe_2NiO_4 is higher at a concentration of 125mg/ml. It is hoped that using these nano-particles compound or pure can combat resistant pathogenic microbes easily and prevent from resistance of other bacteria sensitive to chemical antibiotics.

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