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Research Article

The Study of Synergismic Effects of Iron NanoParticles and Methanolic Extract of *Nasturtium officinal L.* on *Enterococcus faecalis*

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ABSTRACT

The purpose of this study was to investigate the synergistic properties of iron nanoparticles and plant methanol extracts of *Nasturtium officinal L.* against *Enterococcus faecalis* respectively. The iron nanoparticles of 20 nm purchased from Sigma and methanol extract was prepared. Next, dilution of 5, 10, 20 and 40 of NanoIron and concentrations of 20, 30, 50 and 100 mg/ml of the extract was prepared. Finally, a series of dilutions of a combination of plant extracts and Nano Iron were prepared. Iron nanoparticles were more effective than plant extract. Results showed that iron nanoparticles and are methanol extract had synergismic effects.

Key words: Synergism; Iron NanoParticles; *Nasturtium officinal L.*; *Enterococcus faecalis*

INTRODUCTION

Using nanoparticles and their oxide to fight against bacterial infections can be effective as an alternative for antibiotics. Studies have shown that as the size of nano-particles is smaller, they show new and different activities and characteristics. Nowadays, the use of nano-materials is expanding very quickly so that i it is applied in all life aspects, including the fight against disease-causing microbes, diagnosis and treatment of diseases. Nano-materials have shown the lowest toxicity levels in the life cycle and ecosystems. Therefore, the use of these materials can be an appropriate option to fight against germs. In studies conducted, microbial enumeration and bactericidal properties of nanoparticles, such as silver, manganese, iron, titanium, chromium, etc. and their oxide have been proven (kumar, 2007). In recent years, enterococci have emerged as the second leading cause of nosocomial infection and this is due to acquired resistance to several important antibiotics such as beta-lactams, cephalosporins, trimethoprim-sulfamethoxazole and glycopeptides. Urinary tract, bacteremia, endocarditis and meningitis infections are the most important infections caused by Enterococci. Among all enterococci, *Enterococcus faecalis* and faecium are responsible for most infections in hospitals (Frye, 2013). Therefore, the use of antibiotics and natural remedies like herbal extracts and essential oils can be effective against these bacteria. Natural extracts can interfere in

different parts of the cell, including cell wall, cytoplasmic membrane, and cytoplasmic membrane proteins. In addition, they can cause coagulation of cytoplasm contents and leakage of cytoplasmic components and thus prevent the growth of bacteria (Burt, 2004). The aim of this study was to evaluate the synergistic properties of iron nanoparticles methanol extract of nasturtium plant on *Enterococcus faecalis*.

MATERIALS AND METHODS

Iron nanoparticles with dimensions of approximately 20 nm were used in this study. In order to extract, nasturtium plant was collected from meadows and forests of Arasbaran and it was dried at room temperature, away from sunlight. Then, it was powdered by electric grinding and it was extracted by pure methanol solvent. Enterococcus faecalis was also prepared and revived in lyophilized form from Tehran University. The effect of nasturtium extract and nano-iron was evaluated by disc diffusion and dissemination of the wells. First, to determine the effect of iron nanoparticles, dilution series 5, 10, 20, 40 micrograms per ml of nanoparticles were prepared. To determine the effect of dilution, dilution series of 20, 30, 50, 100 milligrams per ml of extract were prepared. To investigate the synergism of property of these two compounds, a compound concentration of above-mentioned dilutions was prepared and it was used against Enterococcus faecalis bacteria (Alizadeh, 2014). To do experiment using method disc diffusion, blank discs were immersed in the considered dilutions so that compounds are absorbed to disc. Then, these discs were dried and used in testing anti-gram. To do experiment using diffusion of the wells, wells with a diameter of 5 ml were created in the Mueller-Hinton agar medium and they were filled by the considered dilutions of the extract. In both of experiments, chloramphenicol antibiotics were used as positive control and DMSO was used as negative control. All plates were examined in terms of inhibition of bacteria and the results were recorded.

RESULTS

The results of above-mentioned experiments are shown in table below.

Table 1: The diameter of inhibition zone using diffusion of well and diffusion of disc to extract of plant

Concentration / Type of compound	20	30	50	100	Negative control	Positive control
Plant extract (Disk)	10	10.32	12	14	-	22
Plant extract (well)	10	10.64	15	16	-	23

* Concentration of extract is based on milligrams per ml.
* The diameter of inhibition zone is based on mm.

Table 2: The diameter of inhibition zone using diffusion of well and diffusion of disc methods for nanoparticle

Concentration / Type of compound	5	10	20	40	Negative control	Positive control
Nano iron (disc)	-	5	12	15	-	22
Nano iron (well)	3	10	15	18	-	23

* Nano-particle concentration is based on micrograms per ml.
* The diameter of inhibition zone is based on mm.

Table 3: The diameter of inhibition zone using diffusion of well and diffusion of disc method for compound concentration

Concentration / Type of compound	Concentration 1	Concentration 2	Concentration 3	Concentration 4	Negative control	Positive control
Compound concentration (disc)	10	14	19	21	-	22
Compound concentration (well)	12	17	21	22.64	-	23

* Concentration 1: 5 mg of nanoparticle + 20 mg of extract
* Concentration 2: 10 mg of nanoparticle + 30 mg of extract
* Concentration 3: 20 mg of nanoparticle + 50 mg of extract
* Concentration 4: 40 mg of nanoparticle + 100 mg of extract
*The diameter of inhibition zone is based on mm

Results of these tables show that both compounds have significant effect on bacteria. After combination of prepared dilutions, the diameter of inhibition zone increased sharply so that it was almost equal to chloramphenicol antibiotic in higher dilutions.

DISCUSSION

Nanoparticles were discussed in two metal and non-metal parts in past days. Metal nanoparticles are being used in pesticides and herbicides for many years. Some of nanoparticles are considered as a modern approach in pharmaceutical development that due to high potential, they are used widely in environmental and pharmaceutical studies (Hardman, 2005). Several reports have been obtained on the antibacterial effects various plants extract from different parts of Iran that these studies have been conducted on various microorganisms (Fazli, 2003). The results showed that the extract of nasturtium plant had inhibitory effects on selected bacteria using disc and well methods, while the diameter of inhibition zone in disc diffusion method was slightly more than diffusion of disc. By examining Table 2, it can be said that nano-iron, as extract, showed inhibitory effect in both methods on the diameter of zone in well was more than disc. One of the reasons for different effects in using these two methods can be due to lack of complete absorption of dilutions by blank disc. As can be seen from Table 3, these two compounds have synergistic properties and diameter of zones increased dramatically, so that in higher concentrations, inhibition zone was almost equal to the positive control. The compound concentration effects were also greater in well method than disc method. By comparing data of Tables 1 and 2, it can be concluded that iron nanoparticles had higher effect than nasturtium extract. Studies conducted by Alizadeh et al in 2014 indicate that iron nanoparticles have inhibitory effect on the growth of Bacillus cereus and Pseudomonas aeruginosa and they prevent the growth of bacteria (Alizadeh, 2014). The results of the study are consistent with the current study results and the current study suggests that iron nanoparticles have antibacterial properties against *Enterococcus faecalis*.

CONCLUSION

It can be concluded that the nanoparticles could be a new method to fight against germs without increasing concern on bacterial resistance. It is recommended that toxicity of these nanoparticles to be minimized by doing some experiments so that clinical experiments can be started for this compound.

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