

Investigation of Eucalyptus and nanosilver as a new nanomixture for growth inhibition of *E.coli*

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ABSTRACT

Nanotechnology is a new and specific method for therapy of diseases. Nanosilver particle is one of the functional fields of nanotechnology. It has been shown that these nanoparticles have antibacterial and antifungal properties. Different studies have been shown that nanoparticles may have an important role in medicine. For example, it has been shown to have inhibitory effects on the treatment of many diseases. The shape and size of nanoparticles are very important. In this study the inhibitory effects of silver on *E. coli* has been investigated. Nanosilver in different concentrations on inoculated blank antibiogram disk was placed on cultivated nutrient agar environment by 0.5 MacFarland's standard. After the first, second, and eighth day the diameter of disks was measured. It was found that nanosilver in a concentration of 50ppm is more effective than the other concentrations for growth inhibition of *E. coli*. The best time for induction of inhibitory effects on *E. coli* was 3 days post treatment with silver nanoparticles. Investigation of inhibitory effects for nanosilver concentration on *E. coli* is an example of functional effects of new nanobiotechnology. It was shown that eucalyptus and nanosilver has the maximum inhibitory effects on the growth of *E. coli* 1 day post treatment. The results from this study suggest that silver nanoparticles in combination with eucalyptus extracts may be useful for the therapy of human bacterial diseases.

1. Introduction

Nanoparticles have many applications in medicine and biology. There is a lack of information concerning the human health and environmental implications of manufactured nanometals (Bhainsa, 2006). The specific physicochemical properties of nanometals are mainly attributed to high surface area to volume ratio, which potentially results in high reaction

activity. Silver is one of these materials that have antibacterial and antifungal effects. Nano-silver is an effective killing agent against a broad spectrum of gram negative and gram-positive bacteria and antibiotic-resistant strains. In this investigation *E. coli* which is a negative gram bacterium was used as a model. The shape and size of nanoparticles are very important in the property of these particles. For this reason, in this study, *E. coli* was treated with spherical shape and 4 nanometer diameter

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nanosilver particles. Nanosilver antibacterial effects have been investigated in many studies. It has been suggested that the resultant structural change in the cell membrane could cause an increase in cell permeability, leading to an uncontrolled transport through the cell membrane and ultimately apoptosis. It has also been proposed that the antibacterial mechanism of silver nanoparticles is related to the formation of free radicals and subsequent free radical-induced membrane and DNA damages. Toxic effect of the silver nanoparticles and the silver ions is associated to the concentration of free radicals and rate of oxidative stress reactions. Silver ions move into the cells and lead to the production of reactive oxygen species and damages the cell membrane (Moudgil and Robert, 2006). Here, we study various aspects of nanoparticle physiological effects on *E.coli*. One of the most important, well-known and widespread medicinal herbs is Eucalyptus because of its antioxidant, antibacterial, and antiviral activities that has been used in traditional medicine. Eucalyptus is a family of Myrtaceae. The primitive natives of Australia used the leaves of Eucalyptus to cure the injuries and to relief the fever. Bluish gum Eucalyptus or Australian fever tree is the most well-known species with medical applications. Eucalyptus leaves have eucalyptol (cineole), trineole, ceskoie terpene alcohols, aliphatic aldehydes, isoamyl alcohols. Eucalyptus has been shown to have antibacterial properties and prevents the risk of growth of infectious agents (Arikan et al., 2001; Arikan and Rex, 2000). The aim of this study was to investigate the effects of Eucalyptus extracts and nanosilver on the growth of *E. coli* *in vitro*.

2. Materials and Methods

2.1. Cell Culture

In order to produce the main samples of bacteria, a vial of *E. coli* bacterium was bought from Pastor Institute (Tehran, Iran). The bactericidal experiments were carried out with gram negative bacteria *E. coli* in NA (Nutrient Agar) medium. The nanoparticles were subsequently stored in the containers and serial diluted with distilled and deionizer water. In this study, nanosilver was used in a concentration of 50 ppm. After that, 0.5 McFarland Standard was inseeded to each plate

containing cultivation environment NA (Nakagawa, 1999).

2.2. Disk diffusion test

Bacterial sensitivity to antibiotics is commonly tested using a disk diffusion test, employing antibiotic impregnated disks. A similar test with blank antibiogram disks was carried out in this study. A 10 microliter suspension of nanoparticles (with specific concentration) was added to blank disks and subsequently was dried in an oven for 1 hour. The treated disks were added to nutrient agar that enriched with *E. coli* and was applied uniformly on the surface of a nutrient agar before placing the disks on the plate. The plates were incubated at 35°C for 24 hours. After which the average diameter of the inhibition zone surrounding the disk was measured with a specific ruler. In the next step, inhibitory zones were measured during 1, 2, 3, 6 and 8 day post treatment. The mean and standard deviation (mean±SD) were calculated for each type of nanoparticle and microbial strain (Christoforidis, 2006).

3. Results

In this investigation, the average of disk diameters of *E.coli* have been measured one day after treatment in 50ppm concentration of nanosilver (0.85 ± 0.01 mm) and has been shown to be significantly changed when compared with control groups ($P<0.01$) (Figure 1). In the eucalyptus extract treated group the average of disk diameters of *E.coli* was 0.91 ± 0.05 mm which was significantly changed as compared to control groups ($P<0.01$). It was also shown that eucalyptus and nanosilver has the maximum inhibitory effects on the growth of *E.coli* one day post treatment.

These results show that in the treated with eucalyptus and nanosilver 1 day post treatment there was a maximum inhibitory effects on the growth of *E.coli*. The average of disk diameters of *E.coli* was 1.23 ± 0.51 mm one day after treatment in 50ppm concentration of nanosilver which was significantly different from control groups ($P<0.01$) (Figure 1). These results show the synergic effects of nanosilver and eucalyptus. The optimum time for growth inhibition, was 3 days after treatment in 50ppm concentration of nanosilver and extract

(Figure 2). In the second day after treatment in 400ppm concentration, the average of disk diameter was 2.48 ± 0.39 mm that was significantly different as compared with control groups. The maximum inhibitory effect against *Escherichia coli* bacteria was founded in 400ppm and in different concentration of nanosilver 3 day post treatment.

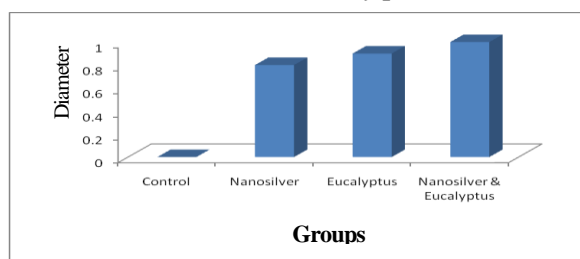


Figure 1. Average inhibitory zone diameter in different groups in 50ppm concentration of nanosilver and eucalyptus

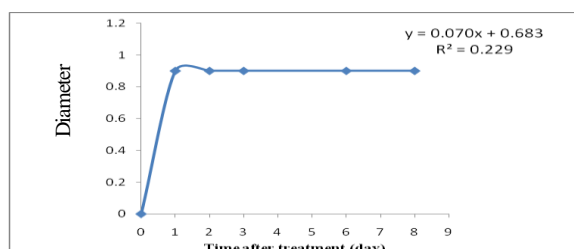


Figure 2. Zone of inhibition diameter against *Escherichia coli* bacteria in different time of treatment in the concentration of 50ppm nanosilver and eucalyptus.

4. Discussion

The results of this study have shown that nanosilver and eucalyptus have synergic effects. The minimum inhibitory concentration (MIC) for nanosilver and eucalyptus was shown to be 50ppm. Moreover the effective time for induction of inhibitory effects on *E.coli* was 3 day post treatment with silver nanoparticles. It has been shown that the size and shape of the particle play a central role in antimicrobial activity of the nanoparticles. In a recent study, colloidal silver particles, with average diameter of 4nm and spherical shape was used for the investigation of antibacterial effects on *E.coli*. Small particles exhibited higher antimicrobial activity than big particles. The small size of silver ions has more interfaces and effects on the cell membrane. It was also shown that the silver nanoparticles of 2.67 nm

protected by hydrogel polymer chains has an excellent antibacterial activity as compared to the larger size silver nanoparticles in the hybrid networks (Moudgil et al., 2006). On the other hand, molecular mechanism provided by silver nanoparticles can be attributed to production of free radicals. These free radicals induce oxidative stress and programmed cell death in *E.coli*. Somayyeh and colleagues in 2011 were found that these free radicals attack the DNA of fungus and bacterium, and cause apoptosis in the cells (Somayyeh et al., 2011). The results from this study show that the best time for induction of inhibitory effects on *E.coli* was 3 day post treatment with silver nanoparticles. Investigation of the inhibitory effects for nanosilver concentration on *E. coli* is an example of functional effects of new nanobiotechnology. The results from this study suggest that silver nanoparticles in combination with eucalyptus extracts may be useful for the therapy of human bacterial diseases. It is also concluded that different size and shape of nanoparticles has different antibacterial properties.

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