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**Research Article**

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# Effect of ZnO NPs on the Growth of *E. coli* in Carrot Juice

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**ABSTRACT**

*Nanotechnology has gained a successful stature as one of vital research areas in the twenty-first century. It has been proven that fixed nanomaterials has a high affinity for interaction with biological macromolecules through bonding to macromolecules, leading to lethal inactivation of bacteria and viruses. This study attempted to examine the effect of zinc oxide nanoparticles on the growth of E. coli bacteria in carrot juice. In fact, it focused on how zinc oxide nanoparticles (ZnO) at concentrations of 1 mM, 3mM, 5mM and 8mM affect the growth of E. coli bacteria in agar medium. Then, a spectrophotometer was used to measure the wavelength of E. coli at concentrations of 3mM, 1mM, 5mM and 8mM followed by dilution. The results showed that E. coli had the lowest absorption coefficient at 5 and 8 mM. Dilution of carrot juice revealed that the number of colonies decreased over time as the lowest number of colonies was observed at concentration of 8 mM within 48 hour. Given the antibacterial properties of zinc oxide nanoparticles, it is a modern technique in the food industry to apply nanoparticles to combat pathogenic microbial agents. It is expected with the application of zinc oxide nanoparticles at allowable doses will be common practice within a few years.*

**Keywords:** Zinc oxide nanoparticles (ZnO NPs); *Escherichia coli*; Carrot juice

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## INTRODUCTION

In recent years, the structure of inorganic nanoparticles has been the subject of speculation in terms of physical, chemical and biological properties. Moreover, the high potential of nanomaterials in biology and pharmacology has attracted many researchers. There are several applications of nanomaterials such as treatment of HIV [1,2]. The nanoparticles can be used in drug delivery programs, production of high quality microscopic images, treatment of cancer and diseases [3]. Moreover, nanotechnology is applied as liposomes for drug delivery to treat cancer. Medical nanotechnology reflects the treatment for leishmaniasis and AIDS [4-7]. Among the most widely used nanoparticles is zinc oxide (ZnO) with a very strong antibacterial activity against Gram-positive and Gram-negative bacteria. These nanoparticles are used as drug carrier influencing the activities of *Escherichia coli* and *Staphylococcus aureus* [8-10]. Zinc oxide nanoparticles can destroy the bacterial cell membrane lipids and proteins. It can be regarded as an antibacterial substance againt gram-positive and gram-negative bacteria [11,12]. The investigation by Nicole Jones et al. on the antibacterial properties of zinc oxide nanoparticles indicated that they hindered the growth of *Staphylococcus aureus* and *Escherichia coli* [13]. Sawai et al. studied the biological effect of zinc oxide nanoparticles, copper magnesium oxide and magnesium oxide, reporting that these nanoparticles have strong antimicrobial effects [14]. Edalatpanah et al. (2015) examined the supplemental effect of zinc oxide nanoparticles and acetic acid against *Staphylococcus aureus* in Grappa, figuring out the antibacterial property of this compound as a supplement [15-17]. Mir-Hosseini et al. (2014) examined the supplemental effect of zinc oxide nanoparticles and citric acid against *Escherichia coli, Staphylococcus aureus, Bacillus cereus* and *Listeria monocytogenes* on Grappa, where the results indicated that these compounds had anti-microbial properties at $P<0.05$. The aim of this study was to investigate the effects of zinc oxide nanoparticles on the growth of *E. coli* in carrot juice.

## MATERIALS AND METHODS

*E. coli PTCC1394* was used in this study. The zinc oxide nanoparticles were supplied by TECONANO with a purity of 99%. At first, the zinc oxide nanoparticles solution was prepared on the concentration 1 mM, 3 mM, 5 mM and 8 mM . They were then they were applied against *E. coli* with 3 replications in agar medium (manufactured by Merck, Germany). At the next stage, spectrophotometry (Iran Tolid medical equipment) was employed to measure the growth rate of *E. coli* at a wavelength of 600 nm in broth medium (Merck, Germany) within 48 hours. Then, the carrot juice was diluted at intervals of 12, 24 and 48 hours at 20°C at concentrations of 5 and 8 mM on an exclusive medium for *E. coli* (manufactured by Sigma Corporation, US) with 3 replications. After 24 hours, the colony-forming unit (CFU) (manufactured by Sigma Corporation, US) counted the number of colonies (Edalatpanah et al., 2014). The data were analyzed through SPSS 21.

## RESULTS

### The effect of zinc oxide nanoparticles on the growth of *E. coli* in agar medium

Table 1 shows the mean and standard deviation of the diameter of formed halo due to addition of ZnO at concentrations of 1mM, 3mM, 5mM and 8mM per millimeters by SPSS. According to Table 1, the diameters of formed halo for concentrations of 1mM, 3mM, 5mM and 8mM were 12mm, 16mm, 20mm and 21mm, respectively.

**Table 1: The diameters of halo formed in zinc oxide nanoparticles at concentrations of 1 mM, 3 mM, 5 mM and 8 mM**

| 8Mm | 5mM | 3mM | 1Mm | Bacteria |
|---|---|---|---|---|
| 21±2mm | 20±1.73mm | 16±1mm | 12±2mm | *E. coli* |

### Absorption coefficient of *E. coli*

Figure 1 displays the absorption coefficient of *E. coli* at 600 nm in concentration of 1, 3, 5 and 8 mM within a 48 hour period. The results showed that concentrations of 5 and 8 mM had the lowest wavelengths which dampened over time.

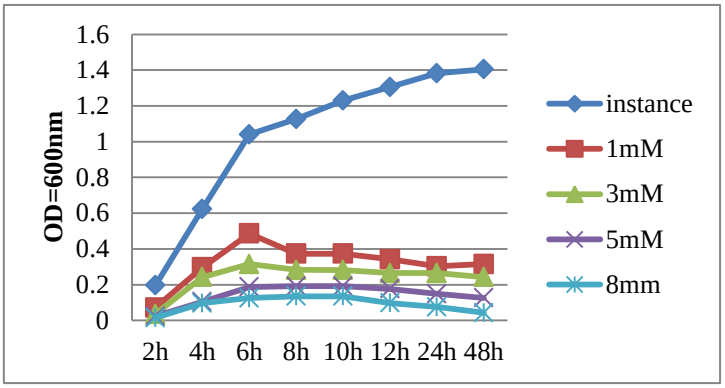


**Figure 1: Growth rate of *E. coli***

### Dilution of carrot juice

Given that zinc oxide nanoparticles at concentrations of 5 and 8 mM had maximum antibacterial properties, both concentrations were applied for dilution of carrot juice. In accordance with Table 2, it took place at intervals of 12, 24 and 48 hours at concentrations of 0, 5 and 8 mM . The results showed that the number of colonies was reduced at 5 and 8 mM over time. In fact, the colonies at a concentration of 5 mM curtailed from 7.21 log CFU/ml to 6.41 log CFU/ml after 12 hours. Moreover, the colonies at a concentration of 8 mM curtailed from 6.84 log CFU/ml to 5.54 log CFU/ml after 48 hours, which was significant at $p<0.05$ (Table 2).

**Table 2: The number of *E. coli* colonies formed in carrot juice (log CFU/ml)**

| | 12h | 24h | 48h |
|---|---|---|---|
| 0mM | 10.54±2.1 | 10.84±3.2 | 11.21±1.9 |
| 5mM | 7.21±1.5 | 6.68±1.7 | 6.41±2.3 |
| 8mM | 6.84±2.2 | 5.89±2.1 | 5.54±2.2 |

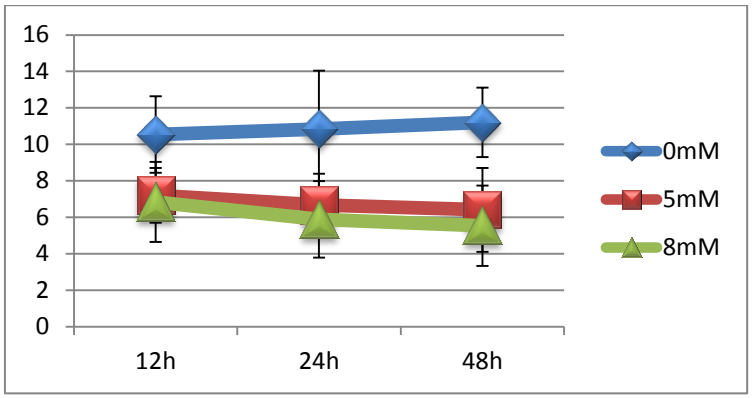


**Figure 2: The number of *E. coli* colonies in carrot juice (log CFU/ml)**

## DISCUSSION

This study attempted to examine the effect of zinc oxide nanoparticles (ZnO NPs) on the growth of *E. coli* in carrot juice. In this study, zinc oxide nanoparticles were first applied in concentrations of 1, 3, 5 and 8 mM against *E. coli*. The results showed that concentrations of 5 and 8 mM had stronger antimicrobial effects than 1 and 3 mM. The absorption coefficient of bacteria at a wavelength of 600 nm was measured by a spectrophotometer. The results showed that *E. coli* at concentrations of 5 and 8 mM had lower growth rates within a 48 hour period at 600 nm. Dilution took place at concentrations of 5 and 8 mM at intervals of 12, 24 and 48 hours. The results showed a decrease in the number of colonies over time. In explaining the findings, it can be argued that zinc oxide nanoparticles reduced the growth of *E. coli* in carrot juice, thus dramatically curtailing the number of colonies. The findings were consistent with those obtained by Edalatpanah et al. (2014) and Mirhosseini et al. (2014) [16,17].

It is expected that nanoparticles will be widely used by the public and the food industries in the next few years. The application of zinc oxide nanoparticles in foods involves one of the substances authorized recently by the Food and Drug Administration. The toxicity of nanoparticles is minimized when the allowed doses are applied.

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