

Analyzing resistance model of *Escherichia coli* bacteria detached from urinary tract infection in the presence of antibiotics and magnesium oxide nanoparticles

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ABSTRACT

Urinary tract infection is the most common problem in health sector. The dominant organism involved in this infection is *Escherichia coli* whose abundance varies in different geographical regions. Keeping in mind the ever-increasing consumption and drug resistance against this bacterium, the current study was conducted to analyze resistance model of the respective bacteria in the presence of antibiotics including: ampicillin, ciprofloxacin, cotrimoxazole, cefotaxime, and magnesium oxide nano-particles. In order to separate the respective bacterial species in a sectional experimental test, the completely randomly selected urine samples were tested using standard methods and 214 samples were reported positive in terms of presence of *E. coli*. Microbial sensitivity analysis was performed in the presence of nano-particles and antibiotics in Mueller-Hinton agar growth medium and the results were analyzed at significance level by means of ANOVA software. In the present research, *E. coli* bacteria exhibited the highest resistance against ampicillin antibiotic (80%) and the lowest resistance against ciprofloxacin (22%). The maximal and minimal effectiveness of nanoparticles of nanoparticles were respectively achieved at concentrations of 0.3 mg/ml (93%) and 0.08 mg/ml (83%). Results showed that drug resistance is increasing in the respective bacteria and appropriate solutions must be devised for prevention. Taking into account their antibacterial potential, nanoparticles are suitable substitutes for antibiotics.

Key words: *Escherichia Coli* Bacteria, Microbial Resistance, Magnesium Oxide Nanoparticles

INTRODUCTION

Urinary tract infection is one of the most prevalent problems in health sector [1]. These infections are among the most common bacterial infections in adults, and particularly, in children. According to reports, 5% of girls and 3% of boys suffer from such infection during childhood [2]. Urinary tract infection holds the second rank after respiratory system infections, and based on the reports, these infections annually involves 150 million people around the world [3]. Numerous organisms are involved in generation of urinary tract infection differs in different regions of the world [4]. Based on the formerly conducted researches, relative abundance of this bacterium causes urinary infections of around 90.6% in Russia [5] and 67.46% in Turkey [6]. Also, the virulence has been reported equal to 67.5 in Kerman City [7]. The antimicrobial medication is essentially based on selective toxicity, and at present, the only way to cure such infections is application of highly efficient but inexpensive antibiotics [8]. Resistance of the respective bacteria

against many antibiotics is the critical problem ahead the medical society. This resistance might have resulted from different causes but the main factor is genetic changes [9]. Increase in travels, population growth, and excessive consumption of antibiotics are among other causes that help expansion of resistant strains [10]. In fact, increase in antibiotic administration antibiotics is directly linked to generation of resistant strains. Furthermore, improper prescriptions of non-planted as well as result of antibiogram test could further lead to generation and development trend of resistant strains [11]. With regard to the role these bacteria under take in generation of urinary infection and its resistance and also taking into account antibacterial potential of nanoparticles, the present research will analyze resistance model of the respective bacteria in the presence of antibiotics including: ampicillin, ciprofloxacin, co-trimoxazole, cefotaxime, and magnesium oxide nano-particles.

MATERIALS AND METHODS

2.1. Reagents

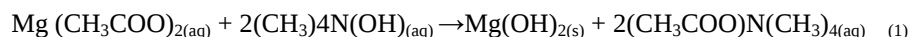
0.14 molar magnesium acetate ($\text{Mg}(\text{CH}_3\text{COO})_2$), polyvinyl pyrrolone ($(\text{C}_6\text{H}_9\text{NO})_n$), tri-methyl ammonium hydroxide ($(\text{CH}_3)_4\text{NOH}$), and ethanol ($\text{C}_2\text{H}_6\text{O}$) were used to prepare magnesium oxide nanoparticles. All chemicals were prepared from Merck.

2.2. Apparatus

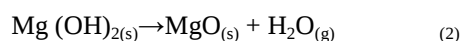
Morphology studies and analysis of synthesized oxide magnesium surfaces were performed using double-beam UV-visible spectrometer(TU 1901), X-ray diffraction apparatus(D/Max-RA) with emission of $\text{CuK}\alpha$, and Transmission Electron Microscope (JEM-200CX).

1.3. Preparation of Magnesium Oxide Nanoparticles

To prepare magnesium oxide nanoparticles, a 50-ml solution of 0.14-molar magnesium acetate was sonicatedfor 30 minutes together with the needed amount of polyvinyl pyrrolone (PVP) as structure-controlling agent. Then, sufficient volume of 0.34-molar aqueous tri-methyl ammonium hydroxide (TMAH) was added to the solution at a slow pace. In this stage, nano-structure of magnesium hydroxide is formed through the following reaction:



At the end of TMAH addition process, the acquired mixture was sonicated for 30 minutes. The precipitated magnesium hydroxide was filtered and washed three times with distilled water and ethanol. 50 ml of ethanol was added to the acquired precipitation and the resulting mixture was sonicated for another 30 minutes, and then, the sonicated mixture was filtered. The final precipitation was dehydrated at 550 °C for 4 hours. The following reaction represents magnesium dehydration stage:



Magnesium oxide nanoparticles were sonicated in ethanol for 30 minutes to eliminate their bulky state. At the last step, the obtained mixture was filtered and dried at 110 °C. The final acquired mixture in powder form is magnesium oxide nanoparticle prepared to be used in the subsequent steps.

The growth medium used in the present study was purchased from Merck Company, Germany. All chemicals used during the experiments of the present research were supplied by Merck Company.

This study was carried out on patients suffering from urinary tract infection, who referred to medical centers under supervision of Shiraz University of Medical Sciences. Sampling from patients was performed using mid-stream method and the samples were transferred to Specialized Microbiology Laboratory inside sterilized vessels for cultivation and detection. Pipette technique was used to culture the samples; 0.05 cc of non-centrifuged urine was removed and cultured on blood agar and eosin methylene blue medium. Quantity of colonies was counted after 24 hours of incubation at 37 °C. The plates with growth of over (10^5) were assumed as positive. Totally, 214 samples were reported as positive in terms of E.coli bacteria. For detection, warm coloring was initially performed on the samples and presence of E.coli bacteria was confirmed after biochemical tests including: coagulase, urease, citrate, sulphured hydrogen production, movement, nitrate reduction, malonate consumption, indole, and fermentation of different sugars and cultivation on MacConkey agar plate. To prepare cellular suspension, nocturnal culture of E.coli on eosin methylene blue plate was removed and transferred to nutrient broth medium. After 18 hours of incubation at 37 °C, concentrations of pipes and McFarland pipe were equalized using spectrophotometer.

1.4. Microbial Sensitivity Analysis

Agar-diffusion technique was applied to determine sensitivity of the isolated bacteria against antibiotics namely ampicillin, ciprofloxacin, co-trimoxazole, and cefotaxime. Mueller-Hinton culture medium was used for this purpose. Two antibiogram tests were conducted for each bacterium: one against the aforementioned antibiotics and the other against magnesium oxide nano-particles at 3 different concentrations.

1.5. Antibiogram Test in Presence of Antibiotic

Antibiotic discs of ampicillin, ciprofloxacin, co-trimoxazole, and cefotaxime were used in the present study. Mueller-Hinton agar growth medium was benefited from for this purpose. Using a sterilized loop, some amount of formerly prepared cellular suspension is taken and cultured on Mueller-Hinton growth medium according to pour-plate procedures. The plates were kept in incubator to be dried at 37 °C for 5 minutes. They were then taken out of incubator and the respective discs were placed on growth medium observing the distance. The plates were kept in incubator at 37 °C for 24 hours. Subsequently, the plates were removed from incubator and diameters of the non-growth rings detected under study light were measured and recorded using vernier caliper. Based on standard tables, the results were reported in three following categories: sensitive, resistant, and intermediate.

1.6. Sensitivity of Separated Bacteria in Presence of Magnesium Oxide Nanoparticle

Initially, three concentrations of the respective nanoparticle i.e. 0.3, 0.08, and 0.15 µg/ml were prepared using deionized water. This experiment was conducted similar to the previous one. The only difference was use of nanoparticles at different concentrations instead of antibiotics. To do so, after culturing bacteria on Mueller-Hinton agar medium and its dehydration, one blank paper of acetate cellulose type was immersed in the desired concentration of the respective nanoparticle suspension with the aid of a sterile forceps, and shortly after dehydration, the blank paper was positioned on Mueller-Hinton agar medium at the appropriate distance. The plates were kept inside incubator at 37 °C for 24 hours. Afterwards, the plates were taken out of incubator and diameters of the non-growth nebula emerged under study light were measured and recorded using vernier caliper. In this case also, the results were reported as: sensitive, intermediate and resistant categories.

RESULTS

3.1. Analyzing Properties of Synthesized Magnesium Oxide Nanoparticles

Diffraction Model of X-ray in the Generated Nanoparticles

X-ray diffraction is caused by a swarm of atoms resulting from amplification of scattered beam in specific spatial directions; following collision of X beam with electrons of a substance, the electrons are oscillated and cause emission of X beam in their surrounding space at the same frequency of the primary beam [12]. If the scattered beams are gathered, a resultant wave will be generated, whose amplitude depends on number of electrons and phase difference of the emitted waves. The generated phase difference is dependent on the difference between the travelled paths by the beams. The beams generated by various atoms also have contrast with each other and will have phase difference due to different travelled paths. This phase difference contributes to variation in amplitude of the beam emitted from atom swarm. Since intensity of a beam is proportional to its squared amplitude, the variations in the distances travelled by the beams results in variation of their amplitudes. Therefore, in specific states where amplitudes of beams are aggregated, the beam emitted from the atom swarm is amplified referred to as "X-ray diffraction". To comprehend this phenomenon, it must be noted that the beams diffracted out of an atom swarm attenuate each other in most of the cases owing to absence of appropriate distance followed by failure in summation of amplitudes, and hence, intensity of the final beam will be extremely low. In diffraction apparatus, X beam is emitted from a beam-generating tube onto the unknown sample and intensity of the propagated beam is measured at different angles. Accordingly, the function of diffraction apparatus is to determine the angles at which diffraction phenomena occurs according to Bragg's equation ($2d\sin\theta = n\lambda$) [10]. Figure 1 illustrates the example of X-ray diffraction for magnesium oxide nanoparticles. Diffraction peaks were absorbed at 2θ value. The significant peaks were applied for estimating size of sample particles using Scherrer's equation ($D = K\lambda/(\beta\cos\theta)$) in which K is constant and equals 0.9; λ is wavelength ($\lambda = 1/5418 \text{ \AA}^{-1}$) (Cu Ka), β is full width at the half-maximum of line, and θ is diffraction angle. Size of particle was estimated using peak intensity ratio. For magnesium oxide nanoparticles, size of particles is estimated to be 70 nanometers, and, increase in sharpness of XRD peaks indicates that the particles are texturally crystalline.

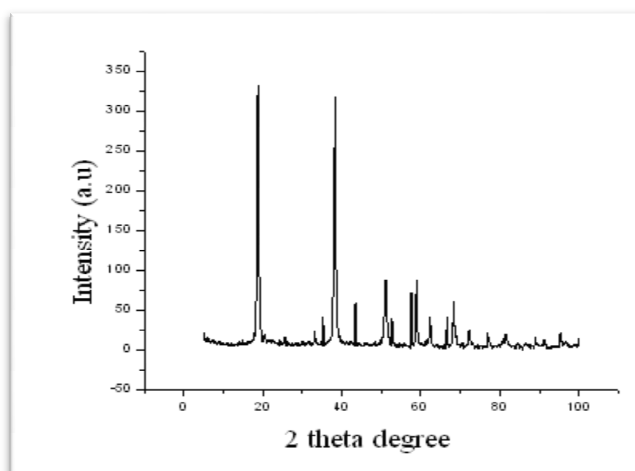


Figure 1: Sample of X-Ray Diffusion (XRD) of Synthesized Magnesium Oxide Nanoparticles

3.2. Properties of Visible-Ultraviolet Spectrum of Magnesium Oxide Nanoparticles

This spectrometry is related to inter-band electron transfers. Such transfers mainly occur between bond orbitals or non-bond electron pairs with anti-bond orbital; as a result, wavelengths of absorption peaks can be correlated to the bonds that are present in the studied species [13]. Figure 2 illustrates UV-visible spectrum of the chemically synthesized nanoparticles. As also demonstrated in the same figure, the absorption peak is in the “300-900”(nm) interval. Non-sharpness of the peak implies generation of nanoparticles at different sizes through this method and UV-visible spectrum responses confirmed the electron microscope data. These data proved specific and quantum properties of nanoparticles.

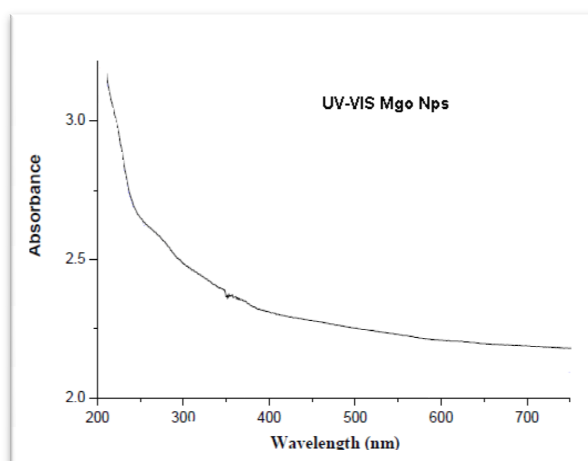


Figure 2: UV-visible spectrum of the synthesized magnesium oxide nanoparticles

3.3. Electronic Microscope Analysis of Magnesium Oxide Nanoparticles

In Transmission Electron Microscope (TEM), the beams are emitted from top to bottom unlike the optical microscopes despite the fact that its function is basically similar to optical microscopes. This electronic microscope is composed of a long column above which the source of electronic beams is mounted. After passing through the sample, the electronic beams collide to a display plane (made of florescent materials) and form the image [14]. Since some beams do not pass through the sample and form black spots, the microscopic images are black & white, and not in color. The sections in Transmission Electron Microscope are prepared much thinner than in electronic microscope and the coloring techniques are also different. Figure 3 shows Transmission Electron Microscope (TEM) image of synthesized magnesium oxide nanoparticles. Due to increase of surface-to-volume ratio with reduction in size of smaller nanoparticles, these nanoparticles are able to play a highly crucial role along the immobilization processes. According to the results obtained from Transmission Electron Microscope studies, diameters of synthesized magnesium oxide nanoparticles are approximately equal to 70 nm.

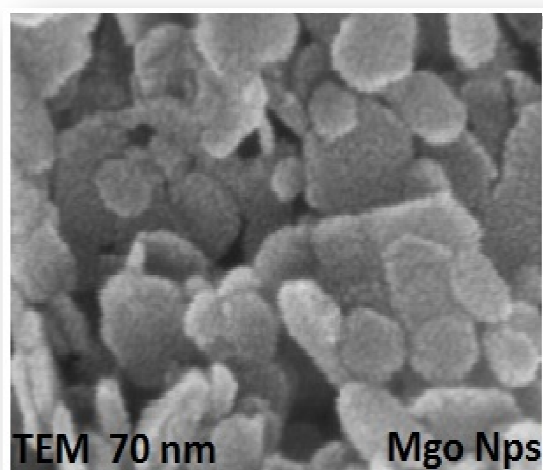


Figure 3: TEM image of synthesized magnesium oxide nanoparticles

Among the patients suffering from urinary infection, 214 samples were assumed positive in terms of presence of *E.coli* bacteria. Table1 summarizes abundances and resistance levels of *Escherichia coli* separated from urinary tract infection against antibiotics: ampicillin, ciprofloxacin, co-trimoxazole, cefotaxime, and magnesium oxide nanoparticles. Also, relative frequency distributions (percentages) of the bacteria separated from the urinary infection are included in Figure 4. According to the results acquired in the present study, the most effective medicine against *Escherichia coli* is ciprofloxacin (77%) and then cefotaxime (72%). Against the respective bacteria, the highest resistance was observed in ampicillin (80%), and then, co-trimoxazole (64%).

Table1: Results of antibiogram test using antibiotics on 214 *Escherichia coli* samples

	intermediate		Resistant		sensitive		
relative frequency	Abundance	relative frequency	Abundance	relative frequency	Abundance	Symbol	Antibiotic
%5.1	11	%80	173	%14	30	AM	Ampicillin
%0.9	2	%22.1	48	%77	163	CIP	Ciprofloxacin
%1.4	3	%64	137	%34.6	74	cot	co-trimoxazole
%1.9	4	%26.2	56	72%	154	CTX	Cefotaxime

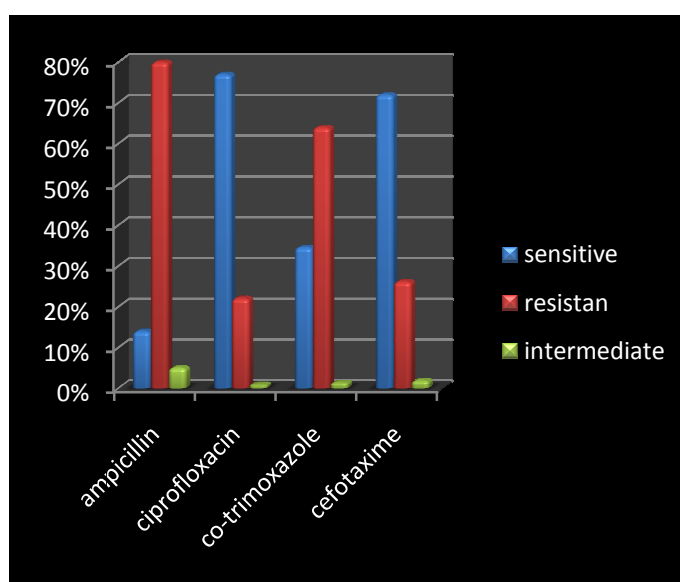


Figure 4: Relative frequency distributions (percentages) against the studied antibiotics

The sensitivity results of *E.coli* separated from urinary system, their frequency and resistance against magnesium oxide nanoparticles are summarized in Table2 and also relative frequency distribution (percentage) of the separated strains against the magnesium oxide nanoparticles are included in Figure 5. The highest sensitivity (88.3%) of *E.coli*

strains was observed against concentration of 0.30 ($\mu\text{g/ml}$). Two other concentrations i.e. 0.15 and 0.08 ($\mu\text{g/ml}$) exhibited lower effects with 88.3% and 83.2%, respectively.

Table2: Results of microbial sensitivity analysis using nanoparticles on 214 *Escherichia coli* samples

	intermediate		resistant		sensitive	
relative frequency	Abundance	relative frequency	Abundance	relative frequency	Abundance	Magnesium Oxide Nanoparticles(concentration
-	-	%6.1	13	%93.5	201	0.3 μg
%0.9	2	%10.7	23	%88.3	189	0.15 μg
%2.3	5	%14.5	31	%83.2	178	0.08 μg

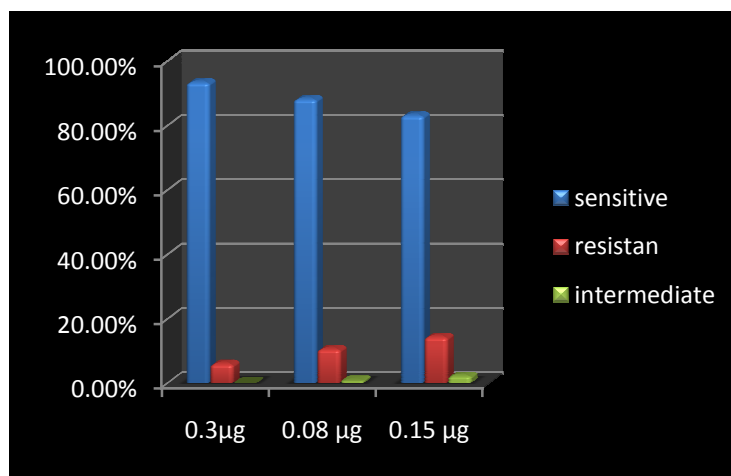


Figure 5: Relative frequency distributions (percentages) against the magnesium oxide nanoparticles

In Figure 6, there is a comparison between effects of antibiotics and magnesium oxide nanoparticles. Based on these results, the concentration of 0.30($\mu\text{g/ml}$) seems to have the largest effect on the respective bacteria. It is also manifested that the smallest concentration 0.08 ($\mu\text{g/ml}$) of magnesium oxide nanoparticle is more effective than the strongest antibiotics in the present study and no remarkable resistance is observed against this amount of nanoparticle.

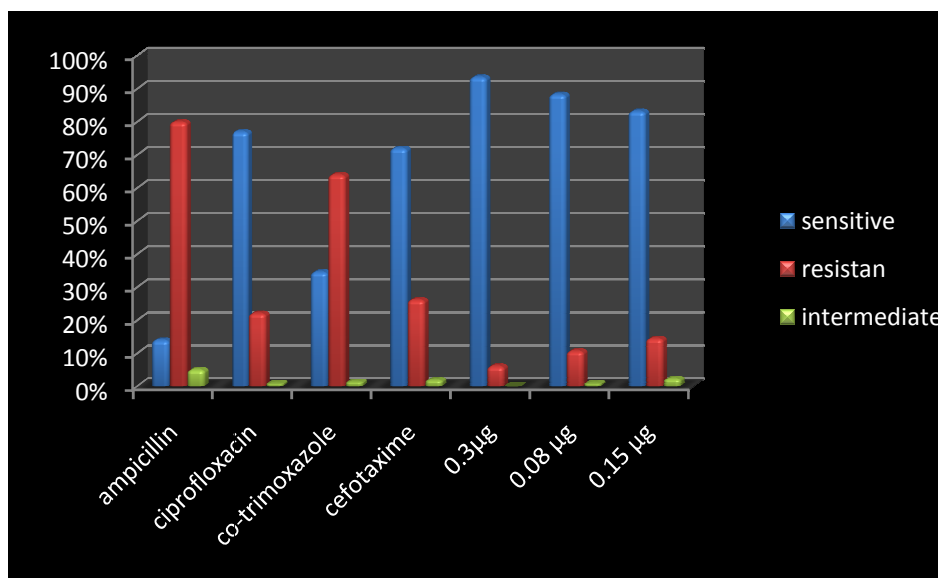


Figure 6 :compares the effect of magnesium oxide and antibiotics

DISCUSSION

E.coli are gram-negative bacteria, regarded as the most virulent cause of urinary tract infection. As of today, various studies have been conducted domestically and globally on resistance model of the respective bacteria against antimicrobial materials. In the study by Mokhtarian Deloei *et al*, the bacteria separated from urinary tract infection

showed the maximal resistance against amoxicillin (100%) and ampicillin (99.1%) antibiotics and the lowest resistance against ciprofloxacin [15]. In the study on *E.coli* bacteria separated from urinary tract infection carried out in Mashhad City, it was reported that the maximal bacterial sensitivity was against amikacin (99.1%), cefixime (97.5%), and ceftriaxone (96%) and the lowest sensitivity belonged to co-trimoxazole (24.2%) [16]. Furthermore, a study conducted in Esfahan City in the same regard corroborates the fact that *E.coli* strains were resistance against ampicillin (72%) and co-trimoxazole (22%) as well as against nalidixic acid (18%) [17]. Through a study in USA, Andrade and *et al*, reported the highest resistance of *E.coli* bacteria separated from urinary tract infection against ampicillin antibiotic (53.6%); thus, the respective bacteria exhibits lower resistance against ampicillin antibiotic in USA compared to the present research. It was also revealed in the same study that the respective bacteria exhibit no resistance against imipenem antibiotic [18]. In the study conducted in Spain by Gupta and *et al*, resistance of *E.coli* bacteria was reported against the following antibiotics: ampicillin (57.3%), co-trimoxazole (25%) and nalidixic acid (20.1%). Compared to the present research, lower resistance is observed in Spain against ampicillin and co-trimoxazole [19]. A similar study was also conducted in India, which indicated *E.coli* bacteria separated from urinary tract infection exhibits the highest resistance against co-trimoxazole (91%) and ampicillin (87%) and the lowest resistance against nitrofurantoin; in comparison with the current study, a higher resistance against co-trimoxazole and ampicillin is observed in India [20]. The results of the present research are in accordance with the studied conducted domestically. But the discrepancy with those conducted outside of Iran is considerable and the microbial resistance phenomenon assumes an ascending trend with further acceleration. Thus, it is vitally necessary to propose and implement an appropriate solution to eliminate the microorganism and prevent from occurrence of microbial resistance phenomenon. During the recent years, organic and inorganic nanoparticles whose structures are characterized by particular physical, chemical, and biological behaviors have widely attracted the attentions. There are reports concerning antibacterial, antiviral and antifungal properties of nano materials, and even, use of nanoparticles has been proposed as a way to cope with AIDS virus [21-22] because nanoparticles which are mainly oxides of heavy metals have high tendency to react and deactivate or immobilize the biological molecules [23-24]. The study by Naghsh *et al* suggested that 40nm spherical silver nanoparticles significantly affect *E.coli* bacteria and concluded that silver nanoparticles can serve as suitable alternatives for antibiotics [25]. In their study, Barzegary *et al* (26) evaluated sensitivity of *E.coli* bacteria against TiO₂ nanoparticles with average size of 60 nm and inferred that TiO₂ nanoparticles at concentration of 0.75% and 1.5% contribute to remarkable reduction in the treatment group compared to the control group. In the present study and compared to other researches, magnesium oxide nanoparticles with average size of 70 nm and crystalline form have remarkable effectiveness on *E.coli* bacteria such that concentration of 0.30 (µg/ml) led to 93% reduction in the tested bacteria. According to findings of the current research as well as other studies, the nanoparticles as metal oxides seem to enjoy favorable antibacterial properties. These nanoparticles cause the bacteria to become slippery in the culture medium through firm connection to outer layer of *E.coli* bacteria and restraining the dehydration process, control of enzymes in the preplasmic space, and control of RNA and DNA [26]. Actually, via conducting further research works in this field, magnesium oxide nanoparticles can be applied as an effective medication in control of urinary infection and other diseases caused by *E.coli* bacteria. This becomes possible through prescribing an appropriate administration formula in combination with other treatments based on their biological effects.

CONCLUSION

Regarding excessive and ever-growing consumption of antibiotics and occurrence of microbial resistance phenomenon and its rising virulence, it is vivid that an effective pharmacological hybrid must be sought for. Nanoparticles can act as suitable alternative for antimicrobial medicines thanks to their antibacterial potentials as well as economic optimality and also confirmation of their high efficiency in numerous research papers.

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