



**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

**EFFECT OF SILVER NANOPARTICLES ON MULTIDRUG RESISTANT
ESCHERICHIA COLI AND *KLEBSIELLA PNEUMONIAE* INDUCED URINARY TRACT
INFECTIONS IN HUMAN AND THEN ON WISTAR RATS**

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ABSTRACT

Bacterial resistance to antimicrobial substances will increase the cost of treatment and cause treatment failure. In this study, the effects of silver nanoparticles on the growth of two multidrug resistant (MDR) Gram-negative bacilli causing urinary tract infections were determined. The side-effects on renal function of male Wistar rats were also assessed. A total of 50 cases with urinary tract infections caused by *Klebsiella pneumoniae* (25 samples) and *Escherichia coli* (25 samples), all of which were resistant to multiple antibiotics were collected and isolated from several hospitals. Later, the bacilli were affected by different concentrations of silver nanoparticles in vitro and the inhibition zone diameter and minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) levels were measured. Then, 32 male Wistar rats were divided into four groups of eight; control group received 0.5 ml normal saline and groups 2, 3, and 4 received 0.5 ml nanosilver with concentrations of 250, 500, and 1000 ppm, respectively. On days 1, 7, and 14, blood urea nitrogen (BUN), uric acid, and creatinine levels were measured. The results of the agar disk diffusion method (30 µg/disc) of silver nanoparticles showed that the highest mean inhibition zone diameter of *K. pneumoniae* was 14 mm. Results of agar well diffusion method of silver nanoparticles (with dilution equal to 1000 ppm) showed that the highest mean inhibition zone diameter of *E. coli* was 18 mm, and the lowest mean was in the bacterium *K. pneumoniae* equivalent to 16 mm. In broth microdilution

method of solution of silver nanoparticles on isolated resistant bacteria, the amount of MIC and MBC in *E. coli* were 33.86 and 63.23 µg/ml, respectively. There were significant differences regarding creatinine and BUN levels between the rats in treatment (1 and 2) and control groups. Our findings indicated that in vitro, between two MDR strains of *K. pneumoniae* and *E. coli* as urinary tract infection causes, the highest sensitivity against silver nanoparticles was related to *E. coli*. On the other hand, in vitro condition, impaired kidney function was observed intravenous rats.

Keywords: Gram-Negative Bacilli, *Klebsiella pneumoniae*, *Escherichia coli*, Multidrug Resistant, Creatinine, Blood Urea Nitrogen

INTRODUCTION

In recent years, drug resistance has significantly increased many pathogenic bacteria, which has made it difficult to treat infections. Therefore, there is a need for safe and effective drugs to treat infections resistant to multiple antibiotics [1]. The inappropriate use of antibiotics in the last two decades has increased the emergence of multidrug resistant (MDR) strains in the community. By providing solutions to decrease the prevalence of colonization of resistant bacteria in hospitals and laboratories and being able to prescribe safe and effective medications for the treatment of infections caused by MDR bacteria, or decrease the resistance among bacteria [2] the problem could be solved. *Klebsiella pneumoniae* and *Escherichia coli* are the most important hospital pathogens. They are the cause of potentially severe infections (like urinary

tract infections), especially in intensive care units [3].

Silver nanoparticles due to their dramatic antimicrobial effect and their increasing use in industries such as health and beauty, catheters, disinfectant sprays, detergents, and toothpaste have become the most widely used particles; this daily use has increased the need for examining these particles regarding their healthy [4]. The size of the nanoparticles is about 3–100 nm, and because of different measurement methods, such as optical absorption, fluorescence, Raman scattering, and magnetic force and electric, they are good indicators for designing biosensors. These particles are used in biological sciences and medicine in biosensors, catalysts, detection of DNA, proteins, and microorganisms, etc [4].

Although the mechanism of bactericidal effect of silver nanoparticles is not

completely clear, but it is possible that silver ions (Ag^+) cause damage in the cell membrane of bacteria [5].

A large group of MDR bacteria produces beta-lactamase enzymes; during recent years, numerous outbreaks of extended-spectrum beta-lactamase-producing organisms have been reported worldwide. This type of outbreak is a serious threat in the treatment of infections [6].

Numerous studies on the antibacterial activity of silver nanoparticles indicate that in the presence of silver nanoparticles in the minimum inhibitory concentration (MIC) of 10 μg , it shows greater antibacterial activity than some antibiotics. Researchers suggest that regarding nalidixic acid, silver nanoparticles show synergistic effect on the antibacterial activity against a number of bacterial strains. These results confirm that the silver nanoparticles have the same ability as antibiotics such as beta-lactams, cephalosporins, aminoglycosides, glycopeptides, and macrolide [7]. Some bacteria, such as *K. pneumoniae* and *E. coli*, have a potential mechanism for degradation of the beta-lactam ring of these drugs [8].

The purpose of this study was first to determine the prevalence of MDR Gram-negative bacilli causing urinary tract infection in patients from several hospitals; secondly,

to investigate the antibacterial effect of silver nanoparticles on these bacteria; and finally, to obtain the minimum effective concentration of silver nanoparticles on the mentioned bacteria in vitro.

METHODS AND MATERIAL

Clinical samples were collected from patients hospitalized with urinary tract infections in various departments of several hospitals in Iran.

Identification of Strains

Isolated strains from patients were identified using biochemical tests. Standard bacterial strains obtained from Scientific and Industrial Research Center of Iran were prepared as lyophilized vials and included standards strains *K. pneumoniae* PTCC 1270, and *E. coli* PTCC 1290. All culture used in this part were produced by Merck Company, Germany.

Agar Disk Diffusion Method

First, bacterial suspension equivalent to 0.5 McFarland standard was provided. Then, on the surface of medium, nutrient agar (NA) was spread in four directions, and then the used discs containing silver nanoparticles, that were soaked with the dilution studied (30 $\mu\text{g}/\text{disc}$) and were dried, were placed with 40 mm distance from each other on plates containing NA medium inoculated with the studied bacteria. After 24, 48, and 72 h,

incubation was performed at 37°C and the size of the zone of growth inhibition was studied. This approach was used to investigate the effect of antibacterial antibiotic discs [9].

Agar Well Diffusion Method

In this method, after the release of bacterial suspension equivalent to 0.5 McFarland standard on the surface of nutrient agar media in four directions, wells using sterile Pasteur pipetting with 6 mm diameter and distance of 40 cm were prepared (in each plate 2 wells, one to inject a certain concentration of silver nanoparticles, and the other for sterilized physiological serum for positive control). Concentration of 100 µl of silver nanoparticles was inoculated in the related well. It is worth noting that the same amount of sterilized physiological serum was injected into the other well. Regarding interpretation of results exactly like the agar disk diffusion, incubation for 24, 48, and 72 h at 37°C was performed and the zone of growth inhibition around each well was measured using a millimeter ruler [9].

Macro-Dilution Method

In macro-dilution method to determine the MIC of silver nanoparticles, Hinton broth dilution series was prepared. For each dilution, 1 ml of bacterial suspension was added. Positive (culture medium containing

bacteria without silver nanoparticles) and negative control tubes (culture medium without bacteria and without silver nanoparticles) were prepared. Finally, inoculated tubes were placed at 37°C for 24 h. After incubation, the tubes were examined for turbidity caused by bacteria inoculated. The lowest concentration of silver nanoparticles that no opaque was observed in it was considered as MIC. To determine minimal bactericidal concentration or minimum bactericidal concentration (MBC), the first transparent tube and one tube before and after it was cultured in nutrient media. Medium inoculated were incubated for 24 h at 37°C, after heating in the absence of bacterial growth, the antibiotics concentration was considered MBC or the minimum lethal concentration of the bacteria (bactericidal) [9]. All laboratory procedures used were performed 3 times. All media used were manufactured by Merck, Germany.

Preparation of Silver Nanoparticles Solution

A volume of 250 ml of silver nanoparticles from Neutrino Tehran Company, which was commercially provided from Spain, with the following specifications: 10–15 nm diameters, purity of 99.9%, and concentration of 1000 ppm was bought. To ensure the accuracy of nanoparticles with the above

identification, 1 g of the nanoparticles was sent to the Department of Materials Engineering of the Islamic Azad University of Najaf Abad. And this center with X-ray experiments confirmed the authenticity of the nanoparticle size and diameter. The electron microscopy image transmission electron microscopy (TEM) (TEM Microscope Model H600, Manufacturer: Philips) of the nanoparticles, including X-ray diffraction pattern of the nanoparticles is shown in **Figure 1**. The silver nanoparticle had the characteristics given in brackets of **Figure 1** caption.

From the original stock solution of silver nanoparticles with concentration of 1000 ppm, serial dilutions were prepared and the obtained concentrations were 250, 500, and 1000 ppm. Blank discs were placed for 1 h in 20 ml of colloidal solution of silver nanoparticles in the mentioned concentrations. After drying, the discs were placed on Mueller Hinton agar medium containing bacterial suspension equivalent to 0.5 McFarland of MDR bacteria causing urinary tract infection and the following standard bacteria: *E. coli* 1270, 1402, *K. pneumoniae* 1290. In addition to the three mentioned concentration of silver nanoparticles solution, a disk impregnated

with physiologic saline as control was placed in the above mentioned environments [10].

Animal Preparation and Blood Sampling

This experimental study was performed on 32 Wistar male rats. The animals were purchased from Shahrekourd University of Medical Sciences and were kept for 2 weeks in the nest for test preparation at Islamic Azad University Felavarjan branch. The animals were kept in proper conditions and temperature (temperature of $2 \pm 22^{\circ}\text{C}$) as well as sufficient lightening (12 h light and 12 h darkness). The animals mean weight was 225 ± 25 g and were divided into four groups of eight. The categories included first group: control group, 0.5 ml of physiologic serum was injected intra peritoneal. Hence, the effect of the shock regarding injection was identical in treatment and control group. Second group received 0.5 ml of silver nanoparticle with 250 ppm concentration injection. Third group received 0.5 ml of silver nanoparticle with 500 ppm concentration injection. Fourth group received 0.5 ml of silver nanoparticle with 1000 ppm concentration injection. The injections were performed for 7 consecutive days. Blood samples were taken from rats during days 1, 7, and 14 after treatment. The blood was taken from the corner of the eyelids of animals with the help of capillary

tube. The sample was centrifuged for 15 min (3000 RPM/min), and serum was separated. Biochemical factors blood urea nitrogen (BUN), uric acid (UA), and creatinine using biochemical kits and auto analyzer (Hitachi Automatic Analyzer 902, Roche) were measured.

Statistical Analysis

To assess statistical data SPSS software version 19 was used. All results were calculated as mean \pm SD. Significance level $P < 0.05$ was considered for all analysis. General linear models with MANOVA methods were used to compare the impact of nanoparticles at three different levels on BUN, creatinine, and UA factors compared with the control group.

Finally to effect on the available eukaryotes in in vitro condition, intra-peritoneal injection of different doses of silver nanoparticle with concentrations of 250, 500, and 1000 ppm on male Wistar rats was done. Moreover, its effect on renal function (measured by urea, UA, and creatinine) and texture, after 7 and 14 days of treatment, were studied.

RESULTS

In this study, 50 clinical samples from two species and genus of *K. pneumoniae* (25 samples) and *Escherichia coli* (25 samples) MDR were collected from three different hospitals over 6 months, and were studied.

Isolates from urinary tract infections had a frequency of 71% (n = 50) (**Table 1**).

All clinical strains *K. pneumoniae* and *E. coli* isolated from urinary tract infections, as well as their standard strains compared with antibiotics of erythromycin, tobramycin, amoxicillin clavulanate, ampicillin, cefotaxime, amoxicillin, tetracycline, cotrimoxazole, vancomycin, cefixime, cloxacillin, cephalixin, and ciprofloxacin were studied (**Table 2**). Standard samples of *K. pneumoniae* and *E. coli* were susceptible to most antibiotics, and in two cases they were semi-susceptible. Therefore, after 3 times of testing, the resistance of the clinical samples of these bacteria was quite different from standard samples. The results of the comparison between the two strains in table 2 shows that *K. pneumoniae* clinical samples had higher levels of antibiotic resistance compared to *E. coli* (**Table 2**).

After placing disc containing 30 mcg of silver nanoparticles onto NA medium and inoculating it with MDR clinical isolates, standard bacteria being examined and incubation at 37°C for 24 h it was observed that the diameter of inhibition of these bacteria compared to the discs were less than the wells method that the same amount of nanoparticles were used. Furthermore, blank discs containing physiologic serum as a

positive control for both standard and clinical strains of bacteria were used (**Table 3**).

According to **Figure 2**, which shows the results of welling in agar plates, the diameter of inhibition in the two bacterial strains is increasing with increasing concentration of silver nanoparticles. This means that by increasing the amount of nanoparticles the influence also increases. Standard strains of bacteria, *K. pneumoniae* and *E. coli* compared with different concentrations of silver nanoparticles had very little resistance and showed high sensitivity.

In the mean MIC and MBC of silver nanoparticles, between clinical and standard strains of bacterial species mentioned, no significant differences were observed. Table 4 shows the results of mean MIC and MBC of silver nanoparticles with the macro-dilution method in minimum required dilution clinical samples based on $\mu\text{g/ml}$.

Findings from the impact of silver nanoparticles injected intra peritoneal in Wistar male rats showed the following results: **Table 5** shows the P value of Dunnett's test (comparing the relevant factors according to different levels of nanoparticles compared with the control group with regard to intervention). Last column of the table corresponds to P value of the comparison of

silver nanoparticle mean at baseline, 7 and 14 days after intervention. If significant, then t-paired test was used to compare the corresponding factor at baseline, 7 and 14 days after the intervention. The P values are provided at the sub-table. According to the results between treatment groups 1 and 2 (Ag 250, 500 ppm) and the control group, a significant difference (decrease) in the amount of creatinine and BUN, was observed respectively, in the 1st week and the 2nd week $P < 0.05$ and for the rest of the treatment groups, there was no significant difference observed ($P > 0.05$). Creatinine levels in the 2nd week had an increase in the treatment groups compared to the control group, but this increase was not significant (back to day one). The amount of urea in the 1st week and the 2nd week in all treatment groups had increased compared to the control group, but this increase was not significant ($P > 0.05$). There was no significant difference observed between treatment groups ($P > 0.05$). The creatinine levels showed a significant difference between the 1st week of the 1st day (before treatment) in the treatment group 1 ($P < 0.05$). Furthermore, the level of BUN, in the 1st week of the 1st day (before treatment), in the treatment group 2 showed a significant difference ($P < 0.05$).

Table 1: Frequency and Percentage of Clinical Samples Studied at Three Different Hospitals

Type and frequency of infection	Urinary tract infections	
	Hospital	Number
Number (1)	30	60
Number (2)	10	20
Number (3)	20	40
Total	50	100

Table 2: The Results of Susceptibility Testing and Drug-Resistant Clinical Isolates of *Klebsiella pneumoniae* and *Escherichia coli* Based on Percentage

Antibiotic	Bacteria			
	<i>Klebsiella pneumoniae</i>		<i>Escherichia coli</i>	
	Resistant	Sensitive	Resistant	Sensitive
Erythromycin	92.20	7.80	98.23	1.77
Tobramycin	52.31	47.69	28.39	71.61
Amoxicillin clavulanate	80.22	19.78	24.56	75.44
Cefotaxime	84.05	15.95	19.97	80.03
Amoxicillin	76.30	23.70	12.69	87.31
Tetracycline	52.94	47.06	64.36	35.64
Cotrimoxazole	60.56	39.44	88.74	11.26
Vancomycin	96.14	3.86	98.87	1.13
Cefixime	88.02	11.98	48.64	51.36
Cloxacillin	97.57	4.43	79.35	20.65
Cephalexin	83.68	16.32	47.87	52.13
Ciprofloxacin	51.69	48.31	47.54	52.46
Ampicillin	88.02	11.98	76.12	23.88

Table 3: The Results of Sensitivity Testing of Clinical Samples of MDR Against Discs of Silver Nanoparticles
P ≤ 0.05

Clinical samples	Mean diameter zone (mm)	
	Disks containing 30 µg of silver nanoparticles	Blank Disc containing physiology serum (positive control)
<i>Klebsiella pneumoniae</i>	9.58	Full growth
<i>Escherichia coli</i>	10.15	Full growth
MDR: Multidrug resistant		

Table 4: The Results of Mean MIC and MBC of Silver Nanoparticles with the Macro-Dilution Method in MRD Clinical Samples Based on µg/ml

Clinical samples	MIC	MBC
<i>Escherichia coli</i>	33.86	63.23
<i>Klebsiella pneumoniae</i>	21.90	41.51
MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration; MRD: Minimum required dilution		

Table 5: Comparison of Creatinine With Blood Urea Nitrogen (BUN) and Uric Acid (UA) in the Treatment Groups 1, 2, and 3 (Ag 250, 500, 1000 ppm) with the Control Group

	1 st day	1 st week	2 nd week	P (Friedman)
Creatinine				
Ag 250 ppm	0.16 ± 0.61	0.11 ± 0.38*	0.11 ± 0.50	0.002
Ag 500 ppm	0.14 ± 0.51	0.12 ± 0.40	0.19 ± 0.59	0.016
Ag 1000 ppm	0.11 ± 0.54	0.09 ± 0.52	0.21 ± 0.56	0.482
Control	0.09 ± 0.64	0.17 ± 0.68	0.10 ± 0.47	0.050
P1	0.961	<0.0001	0.978	-
P2	0.146	<0.0001	0.329	-
P3	0.290	0.051	0.556	-
*Significant difference with the 1 st day P = 0.001				
BUN				
Ag 250 ppm	1.7 ± 23.8	1.7 ± 24.9	3.3 ± 21.1	0.053
Ag 500 ppm	1.8 ± 24.1	4.0 ± 27.5	3.4 ± 20.3*	0.011
Ag 1000 ppm	1.9 ± 23.4	5.8 ± 26.6	3.3 ± 22.0	0.095
Control	1.2 ± 25.0	4.2 ± 28.0	1.8 ± 25.7	0.114
P1	0.326	0.347	0.015	-
P2	0.599	0.998	0.004	-
P3	0.150	0.836	0.056	-
*Significant difference with the 1 st day P = 0.009				
UA				
Ag 250 ppm	0.11 ± 4.34	0.18 ± 4.31	0.24 ± 4.46	0.393
Ag 500 ppm	0.14 ± 4.30	0.17 ± 4.21	0.12 ± 4.40	0.079
Ag 1000 ppm	0.08 ± 4.29	0.18 ± 4.21	0.14 ± 4.36	0.060
Control	0.11 ± 4.31	0.16 ± 4.21	0.09 ± 4.35	0.422
P1	0.945	0.472	0.340	-
P2	0.992	0.999	0.834	-
P3	0.945	0.999	0.993	-
BUN: Blood urea nitrogen; UA: Uric acid				

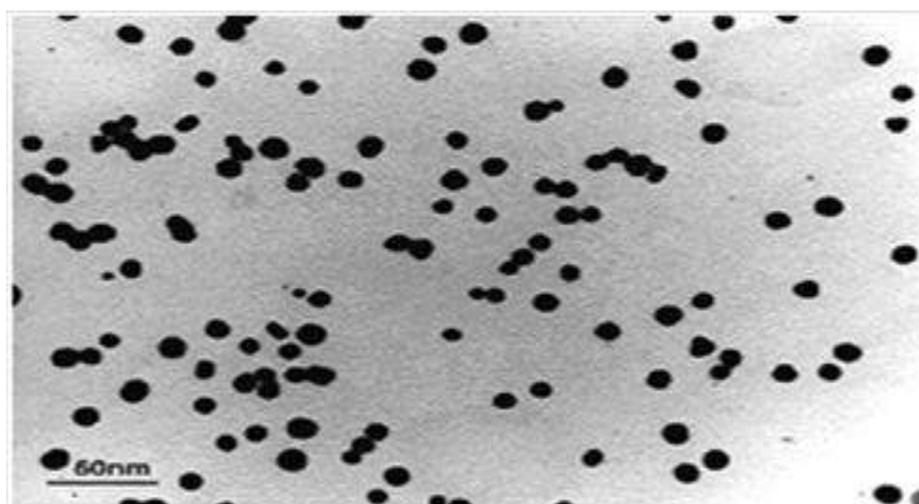


Figure 1: Transmission Electron Microscopy Image of the Silver Nanoparticle (78.8 Anatase, Rutile 21.2 %, Size: 10–15 nm, Specific Surface Area: 100–150 m²/g, Density: 3.84 g/cc)

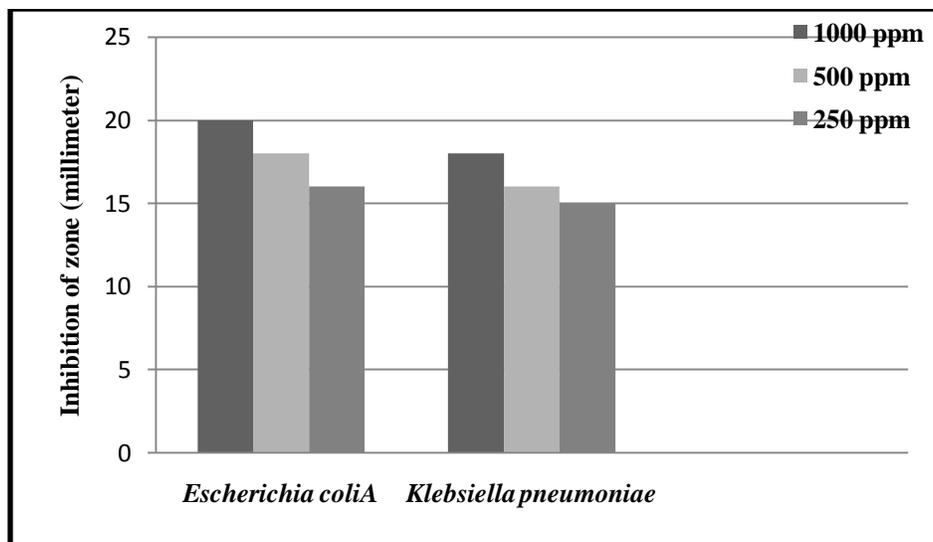


Figure 2: The Mean Inhibition Diameter of Clinical Samples of Multidrug Resistant Against Diluted Solution of Silver Nanoparticles in ppm

DISCUSSION

Findings from this study showed that the highest levels of resistance to antibiotics is related to the bacteria causing infections *K. pneumoniae*, and the highest resistance to multiple antibiotics (MDR) is related to clinical isolates containing this bacteria. On the other side, among the infections being studied in the three mentioned hospitals, the highest rate of infection was related to urinary tract infection. The results of the comparison between this study and other studies showed that silver nanoparticles due to their antibacterial properties can also treat infectious diseases caused by the bacteria that was investigated [10]. The results of the study conducted in 2005 on the antibacterial coatings of silver nanoparticles showed that the activity was photo-catalytic and it greatly

inhibited bacterial growth. The researchers found that the antibacterial effect was related to features such as small size particles, large surface area, high-energy band and having active sites for catalytic reactions [6]. In 2009, the researchers concluded that the antibacterial ability of silver nanoparticles depended on light response and photo-catalytic activity [2]. In 2010, a number of researchers had investigated the relationship between levels of bacterial binding nanoparticles and observed that nanoparticles inhibit growth and reduce the adhesion of bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* [8]. According to various researchers, the mechanisms to eliminate bacteria by silver nanoparticles is that first the nanoparticles establish a strong

bond with the outer membrane of bacteria and inhibit cell activity, including active transport, periplasmic enzymes activities and dehydrogenase, and finally prevent synthesis of RNA, DNA, and protein and can cause cell lysis [11]. According to the results in accordance with tables and charts it can be stated that the antibacterial effect of silver nanoparticles on two Gram-negative bacteria, standard *K. pneumoniae* and *E. coli* was more than two MDR bacteria isolated from clinical samples of urinary tract infections, and the higher concentration of nanoparticles, the antibacterial effect of nanoparticles also increased. The results of many researches indicated that the sensitivity of Gram-negative bacteria against bacteria killing properties of silver nanoparticles was much higher than Gram-positive bacteria [11]. The results from the present study also confirm these results, and the best justification that can be made in this area is due to the significant difference regarding the cell wall of Gram-negative bacteria and Gram-positive bacteria. Gram-positive bacteria cell wall layer contains 40 peptides and glycans, whereas Gram-negative bacteria have only four layers of peptides and glycans. On the other side, the cell wall of Gram-negative bacteria has a high amount of lipid, but the Gram-positive bacteria accounted for a smaller percentage of lipid.

In the present study, the effect of intra peritoneal injection of different doses of silver nanoparticle with concentrations of 250, 500, and 1000 ppm on renal function (measured by urea, UA, and creatinine) in male Wistar, after 7 and 14 days of treatment showed that between treatment groups 1 and 2 and the control group, a significant difference (decrease) in the amount of creatinine and BUN in the 1st week and the 2nd week was observed ($P < 0.05$). In the rest of the treatment groups, there was no significant difference observed ($P > 0.05$).

Creatinine levels in the 2nd week of the treatment groups compared to the control group had increased, but this increase was not statistically significant (back to day one). This indicated damage caused by silver nanoparticles to the kidney tissue, which was also evident in the histological results. The amount of UA levels in the 1st week and the 2nd week in all treatment groups compared to the control group had increased, but this increase was not significant. In recent years, the production of silver nanoparticles due to their antibacterial, antifungal, antiviral and anti-parasitic activity has notably been used. The use of nanosilver in anti-pregnancy equipment, surgical instruments, bone prostheses, and surgical clothes etc. are examples of the use of these substances in

human life [12]. Because of the increased use of nanoparticles, their toxic effects on humans, animals, and environment should be investigated [13]. Although silver nanoparticles show high biological potential, but some reports have shown that many medical devices that are loaded with nanosilver, can cause the release of Ag^+ which are then transported through the bloodstream and get accumulated in many organs such as liver and kidney and cause death (when exposed to high doses of Ag^+). Dimensions of silver nanoparticles are close to Ag^+ dimensions and in many reports, it has been proven that they can easily be moved in the body. It has been suggested that silver nanoparticles induce the same toxicity as Ag^+ . The mechanism of toxicity caused by them is not known, but this may attribute to Ag^+ released from silver nanoparticles, or the nanosilvers that penetrate through the cell membrane [14].

The inhibitory effects of Ag^+ are due to a number of biological events, such as sticking to the cell membrane, absorbed into the cell wall of Gram-negative bacteria, changes in membrane permeability, production of reactive oxygen species, and inactivating cellular enzymes. These compounds may have a negative impact on health and the environment, and this is the reason for high

toxicity of silver nanoparticles [15]. This substance same as other drugs has side-effects and pathological effects on living tissues. This drug is metabolized in the liver after entering the body and is excreted through the kidneys. Despite the widespread use of nanosilver products, few studies have been conducted to determine the biological effects of silver nanoparticles. The absorbed nanosilvers are bonded with plasma proteins and entered into the cell. The substances are spread in organs such as liver, kidney, heart, lymph nodes, brain, and lungs [16]. Substantial amounts of the nanosilver in the liver after inhalation have been observed [16]. Also, renal excretion of them, through urine has also been discovered [17]. It is identified that absorbed silver through oral consumption enters the body and affects the liver, leading to their secretion into bile [18]. Gopinath *et al.* in 2008 showed that metal nanoparticles can cross the cell membrane and leave severe toxic effects on human health. They concluded in their researchers that nanosilver at higher doses ($> 44 \mu\text{g/ml}$) is necrotic for cells, leading to rapid rupture of cell membranes [19]. Takenaka *et al.* also detected large amounts of nanosilver in the liver and kidney of rats [20].

CONCLUSION

According to the results of this study, it can be concluded that in vitro condition, between two bacteria *K. pneumoniae* and *E. coli* resistant to multiple antibiotics causing urinary tract infection, the highest amount of sensitivity against silver nanoparticles with 10 nm diameter and spherical shape was related to MDR *E. coli*. In vivo condition, these nanoparticles in small amounts had toxic effects on renal factors in Wistar rats and it can be concluded that its use for humans due to its toxicity on kidneys of rats in this study and the toxic results on liver, heart, testicles and ovaries of mice in other studies is not recommended for human consumption (these nanoparticles had spherical shape, 10–15 nm diameter, 99.9% purity, and in vivo condition).

ACKNOWLEDGMENTS

This article was a result from the research project approved by Islamic Azad University of Izeh, and with the financial support of the deputy of Islamic Azad University of Izeh it was conducted in Islamic Azad University of Falavarjan branch. Appreciation goes to all the staff of this academic branch, especially Mrs. Shahsar, Mr. Mousavi, and Mr. Sadeghi who helped us in this research.

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