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Original Research

Combined application of sub-toxic level of silver nanoparticles with low powers of 2450 MHz microwave radiation lead to kill Escherichia coli in a short time

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Abstract

Objective(s): Electromagnetic radiations which have lethal effects on the living cells are currently also considered as a disinfective physical agent.
Materials and Methods: In this investigation, silver nanoparticles were applied to enhance the lethal action of low powers (100 and 180 W) of 2450 MHZ electromagnetic radiation especially against *Escherichia coli* ATCC 8739. Silver nanoparticles were biologically prepared and used for next experiments. Sterile normal saline solution was prepared and supplemented by silver nanoparticles to reach the sub-inhibitory concentration (6.25µg/mL). Such diluted silver colloid as well as free-silver nanoparticles solution was inoculated along with test microorganisms, particularly *E. coli*. These suspensions were separately treated by 2450 MHz electromagnetic radiation for different time intervals in a microwave oven operated at low powers (100 W and 180 W). The viable counts of bacteria before and after each radiation time were determined by colony-forming unit (CFU) method.
Results: Results showed that the addition of silver nanoparticles significantly decreased the required radiation time to kill vegetative forms of microorganisms. However, these nanoparticles had no combined effect with low power electromagnetic radiation when used against *Bacillus subtilis* spores.
Conclusion: The cumulative effect of silver nanoparticles and low powers electromagnetic radiation may be useful in medical centers to reduce contamination in polluted derange and liquid wastes materials and some devices.

Keywords: Combined effect, Disinfection process, Electromagnetic radiation, Silver nanoparticles

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Introduction

Disinfection processes have many applications in different domains, for example in pharmaceutical industries, and in hospitals and health care departments (1). Several methods such as incineration, autoclaving, chemical disinfection, pyrolysis and exposing ionized or non-ionized radiation are utilized to reduce hazardous biological contaminations that generated in healthcare centers (2-4). Chemical disinfection process, using various chemical agents such as ozone and chloramines, has been approved for this purpose and broadly used in many industries (i.e. water treatment process). However, these agents are generally categorized as toxic and carcinogenic materials and can be potentially harmful for human and environment (1,5). Moreover, many resistant microorganisms have become a major concern, for the conventional chemical disinfectants, necessitating to find some new appropriate alternatives (1).

Recently many attempts have been performed to evaluate the antimicrobial effects of some nanoparticles such as gold, titanium, magnesium, zinc, copper, and silver (6-9). Among these nanomaterials, silver nanoparticles (Ag NPs) are being abundantly suggested as potent antimicrobial agents.

Furthermore, Ag NPs have been reported as a novel compound for waste water treatment, purification of water and degradation pesticides in pollution environments (10).

Silver ions have been used widely during past decades in topical and wound bandages to prevent bacterial growth but in recent years, Ag NPs have good chemical stability and antibacterial activity comparing the silver ions and have now been used as potent antimicrobial agent in wound dressing products (11,12). Although Ag NPs exhibit good antimicrobial effects against pathogenic microorganisms but these nanoparticles can cause some toxic effects in the living organisms (13-15) and certainly produce argyrosis disorder in human (12).

On the other hand, use of microwave irradiation to decontaminate medical waste and disinfect medical (i.e. soft contact lenses, plastic laboratory or urinary catheter) are also reported in literature (16). Electromagnetic radiations (EMR) with the frequency range between 300 to 3000 *MHz* are categorized as microwaves radiations. However, 2450 MHz electromagnetic wave are typically generated in microwave oven for different purposes (16). Microwave radiation can increase temperature of biological organs or tissue and accelerate movement of molecules (17). Microwave radiation can also be absorbed by any moisturized materials to induce molecular vibration. The energy produced during molecular vibration is converted to heat which can increase the mortality rate of living microorganisms (16). Integration of this physical treatment with a chemical disinfectant agent such as metal Ag NPs may improve the antimicrobial effect of Ag NPs at sub-toxic concentrations. In the present investigation,, the effect of 2450 MHz microwave radiation along with sub-inhibitory concentration of Ag NPs was investigated for the antibacterial activity.

Materials and Methods

Silver nanoparticles and determination of minimum inhibitory concentration (MIC)

Nanosilver colloid (Figure 1) containing Ag NPs range in size 28 to 122 nm with an average size of 52 nm was synthesized using biological technique, reported previously (18,19). Serial broth dilution method using Müller-Hinton Broth (MHB) was used for MIC determination test. MHB were supplemented with different amounts of Ag NPs to obtain final concentrations of 5, 6.25, 10, 12.5, 20, 25, 40, 50 µg/ml. In next step, the test strains including *E. coli* ATCC 8739) were separately suspended into sterile NaCl solution (108 CFU/ml) to achieve the optical density of 0.08-0.1 at 625 nm

(corresponding to 0.5 McFarland standards). Subsequently, this suspension was further diluted by sterile NaCl solution (one hundred times). Aliquots of this diluted suspension (10 µl) were then inoculated to above MHB to achieve 104 CFU/ml cells. All MHB media were incubated for 24 h at 37 ºC. The data are reported as MIC (the lowest concentration of Ag NPs which inhibited visible growth of the test strain) (20).

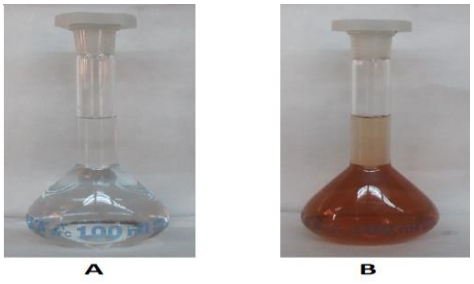

Figure 1. Volumetric flask containing silver nitrate solution before (A) and after (B) adding the supernatant of fresh cultured of K. pneumonia.

Combined effect of silver nano particles with low powers of microwave radiation

Time-kill course assay was applied to evaluate the antibacterial activity of low powers of 2450 MHz electromagnetic radiation (100 W and 180 W) in absence and presence of Ag NPs at sub-inhibitory concentration (6.25µg/ml) against *E. coli*. A microwave oven (SAMSUNG M2330-DN) was used and operated at low powers (100 W or 180 W).Two series of test tubes contain 1 ml of Ag NPs colloid (6.25 µg/ml) were inoculated by *E. coli* (3×10^8 CFU/ml) and separately irradiated in a microwave oven operated by powers of 100 and 180 W for 5, 10, 15 , 20 s. The viable counts of bacteria in all test tubes were determined by standard colony forming unit (CFU) method before and after microwave treatment. For this purpose each sample was diluted with normal saline and was cultured in Müller-Hinton Agar medium (MHA-Merck,Germany). All plates were ncubated at 37 ºC and the grown colonies were counted after 24 h. The results were reported as CFU/ml. The viability of test strain was also studied in the presence of sub-MIc concentration of Ag NPs (6.25 µg/ml) and absence of EMR radiation and served as control. Furthermore, same above time-kill assay was repeated for other test strains which used during this study (Bacillus subtilis spores, multiple resistance Staphylococcus aureus, Candida albicans).

The viability of E. coli in different temperature

Microwave irradiations elevate the temperature of liquid matrix samples so the temperature of the liquid samples were monitored after microwave irradiation and are reported in Table 1. To simulate all temperature caused by EMR radiation and evaluate of different temperature on the mortality of the cells, test tubes contain bacterial suspension (E. coli) were heated in water bath for same exposure time (5, 10, 15, 20 s) which reported in Table 1.

The viable counts of in all water-bath heated samples were determined by CFU method.

All of the test experiments that mentioned in “Materials and Methods” section in this article were carried out in triplicate.

Results and Discussion

Antibacterial assay

Sensitivity of *E. coli* ATCC 8739 against biogenic Ag NPs was determined initially by serial broth dilution method. MIC value against Escherichia coli ATCC 8739 was found to be 10 µg/ml for Ag NPs. A sub-MIC concentration of Ag NPs was selected (6.25 µg/ml) and applied to decrease the time required for killing of E. coli under low powers of 2450 MHz EMR (100 and 180 W). As shown in Figure 2A and B, the Ag NPs increased the lethal effect of 2450 MHz EMR in comparing with the conditions in which no Ag NPs have been applied.After a short exposure time (5 s) the viable counts of E. coli were suddenly decreased from 1×108 to 3×10 2 CFU/ml in normal saline solution which

Table 1. The temperature (^{o}C) after EMR at different time intervals with low powers (100 and 180 W) in two circumstance: absence and presence of Ag NPs.

Time (s)	100 W		180 W	
	In presence of Ag NPs	In absence of Ag NPs	In presence of Ag NPs	In absence of Ag NPs
0	25	25	25	25
5	38	40	40	42
10	39	41	42	45
15	43	45	48	53
20	47	49	52	56

supplemented with NPs and irradiated 2450 Hz EMR in a microwave oven (100 W) (Figure 2A). No viable cells were detected by CFU method in above experiment after a longer exposure times (10 and 20 s). In the free Ag NPs solutions which were irradiated by 2450 MHz EMR (100 W) for 5 or 10 s, the viable counts were determined as 1×10^{8} and 2.87×10^{2} CFU/ml, respectively (Figure 2A). No viable cells were detected in the Ag NPs free suspensions after 20 s irradiation with 2450 MHz EMR in a microwave oven operated at 100 W.

The lethal effects of 2450 MHz EMR with 180 W in presence and absence of Ag NPs were also investigated in this study (Figure 2B). The required time for total destruction of *E. coli* in Ag NPs containing normal saline solutions which irradiated in microwave oven (2450 MHZ/180W) was shorter than the time need (5 *s*) to kill this test strain in the absence of Ag NPs (10 s). The switch of microwave oven from 2450 MHz EMR-/100W to 2450 MHz EMR/180 W cause a considerable increase in lethal effect of EMR in presence or absence of Ag NPs (Figure 2A and B). Also it should be noted that in time kill assay no antibacterial effects was observed for Ag NPs which tested at concentration of 6.25µg/ml in the absence of EMR radiation after short exposure times (5, 10, 15 20 s).

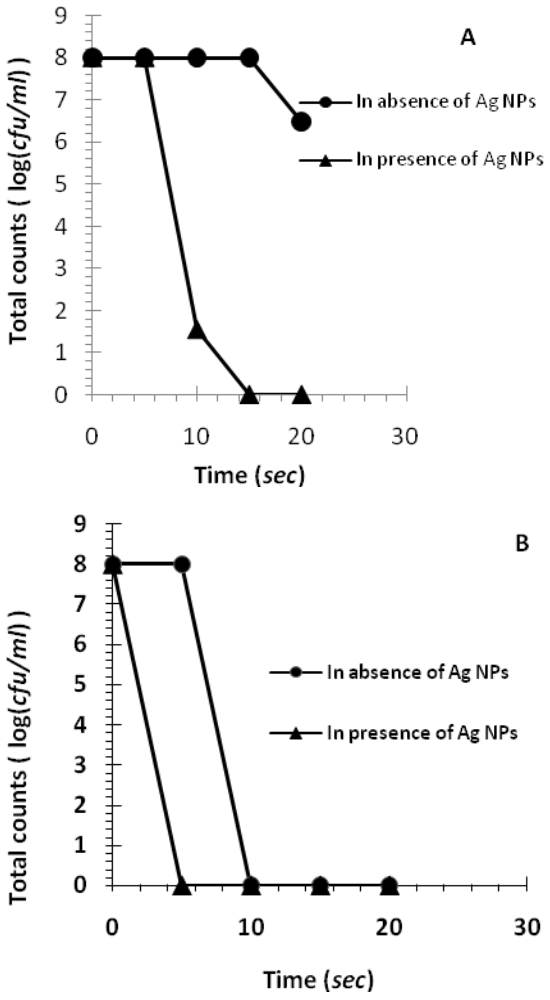

Figure 2. The viability of *E. coli* during treatment with the power of 100 W (A) and 180 W (B) in 2450 MHz microwave oven in the presence and absence of sub-MIC of Ag NPs (6.25 µg/ml) produced by bio-technological process**.**

The survival of E. coli in different temperature

Table 1, shows the temperatures measured in all normal saline solutions before and after EMR radiation process. The temperatures were found to be increasing with the intensity of EMR or Radiation time.

No significant differences were detected between the temperatures recorded in the Ag NPs containing or Ag NPs free samples, irradiated by EMR at both power intensities (100W or 180 W) after different intervals (5, 10, 15, 20 s).

To study whether the increasing temperature is the lone factor forbacterial mortality, many samples of bacterial suspensions were subjected to EMR for 5, 10, 15, 20 s in a bain-marie at the different temperatures and the results are also tabulated. After exposing the cultures for such fixed time intervals aliquots (100 µl) were withdrawn from the test tubes and subjected to CFU assay to determine the viable counts. Preresults obtained shows that no considerable decreases in viable cell counts in all tested samples which warmed for 5, 10, 15, 20 s in bain-marie apparatus (data not shown).

Ag NPs have been used in recent years as antiseptic agents for reducing the hazardous of polluted materials (10). The antimicrobial potential of Ag NPs is much more than antimicrobial activity of soluble silver ions (11, 12). Microwaves are able to decontaminate wastes and disinfect medical devices such as plastic laboratory container (16). In the present study, as a novel approach, the combination effects of Ag NPs and microwave radiation was investigated against *E. coli* by selection of sub-MIC of Ag NPs (6.25 µg/ml) and low powers of EMR (100 W and 180 W) to ensure that the antibacterial effect which obtained after a short time exposure of EMR (5-10 s) was not contributed to each factor alone. Therefore the effect observed combination of Ag NPs and low power of microwave radiation. The temperatures of microbial samples without and with the sub-MIC of Ag NPs (6.25 µg/ml) were immediately measured by a thermometer before and after microwave treatment at different radiation times (5, 10, 15, 20 s).

These recorded temperatures were simulated in bain-marie apparatus. The *E. coli* bacterial suspensions with and without Ag NPs were incubated in these recorded temperatures for the same times which scheduled for microwave treatment (5, 10, 15, 20 s).

No considerable changes in viable counts were detected for all samples incubated in water bath at adjusted temperatures demonstrated in Table 1. The results indicated the existence of a factor more than thermal effect to inflict the mechanisms of the bactericidal effect of low power EMR in the presence of sub-inhibitory concentration of Ag NPs. Ag NPs decreased the required time of EMR for total destruction of test strain in comparing with the control samples which was without Ag NPs.

Further antimicrobial evaluation

The combined effect of sub-inhibitory concentration of Ag NPs and low power 2450 MHz EMR has been observed for other test strains (*Bacillus subtilis* spores, multiple resistance *Staphylococcus aureus*, *Candida albicans*) which were further evaluated (Figures 3 and 4, respectively). The obtained MIC of Ag NPs against both mentioned test strain was 20 µg/ml. Therefore a sub-MIC concentration (10 µg/ml) of Ag NPs was chosen to evaluate the combined effect of Ag NPs and low power 2450 MHz EMR against these new selected test stains.

The Ag NPs increased the lethal effects of 2450 MHz EMR against *Staphylococcus aurous* (MRSA) and *Candida albicans*.

It has also been observed in Figure 3A, that MRSA bacterium shows a good tolerance under 2450 MHz EMR irradiation generated in microwave oven operated at 100 W. However in presence of Ag NPs the mortality of MRSA was significantly increased.

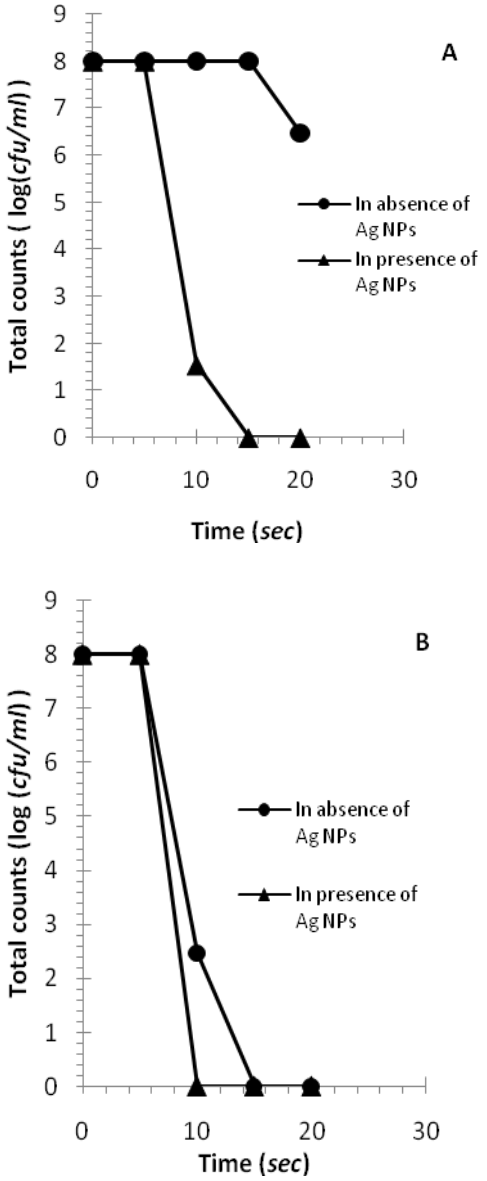

Figure 3. The viability of *MRSA* during the treatment with power of 100 W (A) and 180 W (B) in 2450 MHz microwave oven in the presence and absence of sub-MIC of Ag NPs.

When the spores of *B. subtilis* were also added to tested microbial panel, the effect observed for vegetative form alone could not be observed. These spores are heat resistant germs and their viable counts remain unchanged during irradiation in microwave oven operated with low power energy (100 or 180 W).

As emphasized, the mechanisms of microorganisms destruction by microwaves is not known completely, it can be attributed to the thermal and non-thermal effects (17). The heat generated in microwave oven has different impact on the biological molecules comparing to the head generated in a conventional electric oven.

Microwave induces vibration in molecular level and prolongs the motion of the molecules (17,21,22). Heat has been produced due to vibration of polar molecules such as water molecules and their resistance (2). So by the molecules of water in substances; the waves are absorbed amid the procedure of resonance and the agitation of molecules of water and cause a rise in the temperature of samples (2,23). Ag NPs might enhance the non-thermal effect of EMR and accelerate molecular vibration. Hence the microwave disinfection depends on some factors including the mass and the humidity of the waste, calorific capacity of the materials and the time of radiation and heating (2). Nevertheless, thermal effects such as increasing in local temperature can also add to denaturation of the proteins or disrupt the biologic materials and biomolecules in microorganisms (6).

In the present study biogenic Ag NPs were used to increase the killing effect of microwave. Compared other nanoparticles such as gold nanoparticles, Ag NPs have good antibacterial action and can be used as a disincentive agent (1).

Conclusion

The present investigation describes the improving effect of Ag NPs on the lethal action of EMR generated in a microwave oven. In our previous study, we have reported enhancing effects of gold nanoparticles (Au NPs) on lethal action of EMR in microwave oven (6). However, the present study employs Ag NPs instead of Au NPs because using Ag NPs are more cost effective, common and have more antimicrobial effects than Au NPs. To the best of the authors' knowledge this is a pioneer report on the combined treatment of EMR with Ag NPs for quick killing of some microorganisms, especially *E. coli*, in

a short exposure time. Our study showed that although EMR can be performed to kill

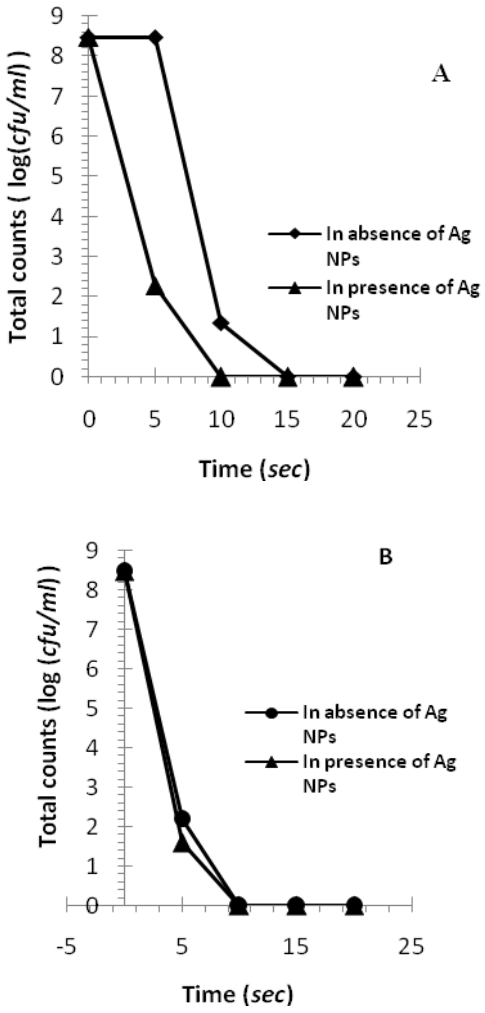

Figure 4. The viability of *C. albicans* during treatment with power of 100 W (A) and 180 W (B) in 2450 MHz microwave oven in the presence and absence of sub-MIC of Ag NPs.

bacteria, the metal nanoparticles when applied with EMR can lessen the time required to deactivate microorganisms. Therefore this method could be used for rapid disinfection of contaminated waste materials generated in medical centers and for sanitization of medical devices such as non-metal dentistry tools or gauze.

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