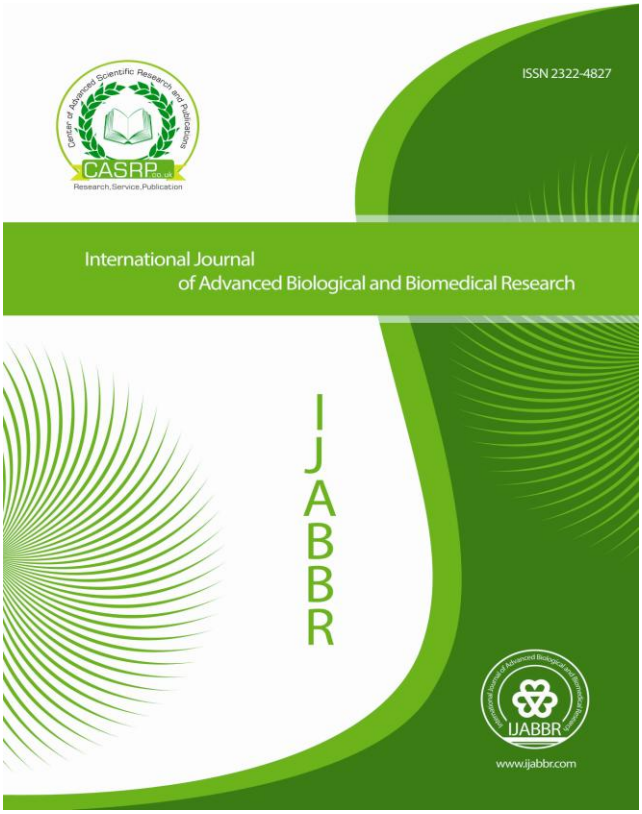

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Original Article **Open Access**

Antimicrobial activities of gold and silver nanoparticles against *Vibrio cholera*

Mahin Rigi*

Department of fisheries, Hamoun International Wetland Research Institute, University of Zabol, Zabol, Iran.

Abstract

Vibrio cholerae is a human pathogen that causes mild to severe diarrheal illnesses and has major public health significance. The aim of this study was to investigate the effects of antimicrobial activity of the gold nanoparticles on *Vibrio cholera*. Gold and Silver nanoparticles are chemically synthesized. Standard strain of *Vibrio cholera*was cultured in a nutrient broth. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was determined by micro dilution.

Keywords: Vibrio cholera, Antibacterial activity, Gola nanoparticles, Silver nanoparticles.

1. Introduction

Nanotechnology is emerging as a rapidly growing field with its application in industrial biomedical and electronic. Nanoparticles of noble metals belong to the extensively studied colloidal systems in the field of nano-science and nanotechnology. Noble metals such as Au, Ag, Pd, Pt and Cu have been widely used for the synthesis of stable colloids which are useful in the areas of opto-electronics, catalysis, photothermal therapy, surface enhanced Raman scattering (SERS) detection and biological labelling. Gold nanoparticles have unique physical and chemical properties such as high stability, high resistance to cold and high ability to absorb and emit light and are synthesized in various sizes and shapes such as spheres, rods, crystal, triangular and spiral. Gold nanoparticles are widely used in medical fields such as DNA diagnosis and cancer treatment, medication delivery, uses in biosensors,

***Corresponding author:** Department of fisheries, Hamoun International Wetland Research Institute, University of Zabol, Zabol, Iran.

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and protein detection and removal of pathogenic microorganisms from contaminated water (Han et al., 2006). Vibrio cholerae is the causative agent of cholera disease which often involves the third world countries. This organism is a Gram-negative bacteriaand belong to the Vybryvnasyh family. Despite the treatment of patients with cholera, by replacing the water and electrolytes disease, it is still associated with high mortality. In areas that do not have good healthcholera is often endemic and patients with immune deficiency, such as children, elder people and travelers are asymptomatic carriers of epidemic in these areas (Vanden et al., 2007; Hill et al., 2006; Chinnapen et al., 2007). Silver ions and silver-based compounds ions are among the inorganic antimicrobial agents which inhibits the growth of microorganisms and shows a strong biological effect against many bacterial species (Costa et al., 2011). Silver has high thermal stability and low volatility so it can withstand conditions during processing (Kumar and Münstedt, 2005). According to a study conducted by Douglas et al. it was suggested that the release of silver ions gradually, and thereby inhibiting the production of ATP and transcription of DNA through direct damage to the cell membrane by silver nanoparticles, the creation of reactive oxygen radicals by silver nanoparticles and silver ions caused antimicrobial activity. Silver ions have the binding ability to the electron donor groups such as sulfur, oxygen or nitrogen in the molecule (Dallas et al., 2011). The aim of this study was to investigate the effects of gold and silver nanoparticles on Vibrio cholera.

2. Materials and methods

Bacterial strain was obtained from standard laboratory. Evaluate the antibacterial activity of the gold nano particles was investigated using strain of Vibrio cholera PTCC 1611. The typed culture of bacterial was sub-cultured on Nutrient agar (Oxoid) and stored at 4 ^{o}C until required for study.

2.1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of gold and silver nanoparticles

The broth microdilution method was used to determine MIC and MBC. All tests were performed in Mueller Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v/ v). Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranged from 6.25 ppm to 100 ppm. To each well, 10 µl of indicator and 10 µl of Mueller Hinton Broth were added. Finally, 10 µl of bacterial suspension (10^6 CFU/ml) was added to each well to achieve a concentration of 10^4 CFU/ml. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then they were placed in an incubator at 37°C for 18–24 hours. The color change was then assessed visually. The lowest concentration at which the color change occurred was taken as the MIC value. The average of 3 values was calculated providing the MIC values for the tested gold and silver nanoparticles . The MIC is defined as the lowest concentration of the gold and silver nanoparticles at which the microorganism does not demonstrate the visible growth. The microorganism growth was indicated by turbidity. The MBC was defined as the lowest concentration of the gold and silver nanoparticles at which the incubated microorganism was completely killed.

2.2. Statistical analysis

All experiments and measurement were repeated at least three times. Statistical analyses were performed using SPSS and Excel 2010 software. All experimental results were analyzed using mean descriptive statistics and the correlation-coefficient. A value of $P<0.05$ was regarded as statistically significant.

3. Results and discussion

The result of gold and silver nanoparticles showed the MIC and MBC against V.cholera was 200 and 50, 100 ppm. Nanoparticles can act as antibacterial and antifungal agents. This is based on binding to the cell surface that change and damage cell structure and cell functions such aspermeability. Also, by creation of cracks and holes, and effect on respiratory chain enzyme activity cause cell death (Zawrah et al., 2011). Chvalybo et al reported that silver nanoparticles, gold nanoparticles, platinum nanoparticles, and metal nanoparticles are harmful for bacteria and fungi. They showed morphological changes result from the interaction between microorganisms and nanoparticles, using electron microscopy. This interaction causes damage to the cells of the fungus. Silver nanoparticles bind to the microbial cell surface. This connection causedstructural changes and cell damage and in particular affect the vital activity of cells such as permeability, respiratory chain enzyme activity and eventually

leads to cell death (Chwalibog et al., 2010). In the study of Adavallan and Krishnakumar, gold nanoparticles (Au-NPs) were synthesized at room temperature by means of *Morusalba* (mulberry) leaf extract as reducing and stabilizing agent .The FT-IR results indicate the presence of different functional groups in the biomolecule capping of nanoparticles. Further, biosynthesized Au-NPs show strong zone of inhibition against *Vibrio cholera* (gram-negative) and *Staphylococcus aureus* (gram-positive) whereas, chemically synthesized Au-NPs and mulberry leaf extract exhibit a fair zone of inhibition (Adavallan and Krishnakumar, 2014).

Naghesh et al. (1392) investigated the inhibitory effects of silver nanoparticles and ethanol extract of eucalyptus on the growth of E. coli. The results showed that the inhibition diameters of E.coli bacteria on the sixth day was 5.0 mm in the control group and in the experimental groups with silver nanoparticles concentrations of 1.3, 25.6, 12.5, 25 and 50 ppm plus 100% ethanol extract of Eucalyptus were 55/0, 58/0, 82.0, 83/0 and 02/1 mm respectively. This difference in 12/5,25, and 50 ppm silver nanoparticles compared to the control group was significant (Naghesh et al., 1392). The studies conducted by Soltani and colleagues demonstrated that silver nanoparticles can control the fish pathogenic bacteria, including strains of Streptococcus iniae, Lactococcusgarvieae, Yersinia rukeri, Aeromonsshydrophila, and also fugues Saprolegnia which caused losses in incubation intestines of fish spawn to a large extent (Soltani et al., 2011; Oltani et al., 2009).

Kalbasi and colleagues studied the effect of colloidal silver nanoparticles on the intestinal bacterial flora of rainbow trout population. The results showed that lactic acid bacteria, Mesophilic and Enterobacteriaceae of fish's intestinal as well as Mesophilic and cold-like bacteria of aquarium water statistically haven't shownto receive any significant effect of the silver nanoparticles. The fish's intestine in silver nanoparticles 1ppm treatment were more cold-like population (6/49±./02 logcfu/g), than control group (5/72±./17 log cfu/g).

Krishna Raj and colleagues reported that the minimum inhibitory concentrations of silver nanoparticles on both standard sides of coli and Vibrio choleraewas 10 micrograms per mL (Krishnaraj et al., 2010). Asadi and his colleagues in 2014, with the use of synthesized silver nanoparticles chemically, reported that the inhibitory concentration of Bacillus cereus bacteria was 20 micrograms per ml (Asadian et al., 2014).

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