Efficiency of silver nanoparticle against virus coronaviruses

Ali Imjayyid Hashim¹, Dr. Jasim Abdulaziz Al Falahy², Prof. Dr. Suzan Saadi Hussain³, Assist Prof Dr. Nihad Khalawe Tektook⁴*

¹²³Mustansiriyah University-College of Science; ⁴M.B.ChBDGSFRCS (Technical Undersecretary of the Ministry of Health and Environment)
⁴MiddleTechnical University-College of Medical and HealthTechnology- Medical laboratory techniques dep -Iraq
*Corresponding author: drnihadkhalawe@gmail

ABSTRACT

Background: currently know about the Flu (Influenza) and the COVID-19 (Coronavirus) disease, which we continue to learn more about, both may present issues for the very young, the elderly, and those with underlying medical conditions. The current annual vaccines and effective antiviral drugs are not available sufficiently. Therefore, aim in current study used particles of silver as general antiseptic and disinfectant in order to prevent spread of infectious agents especially viruses. Material and methods: Maintenance of cell cultures: To determine the cytotoxic effect of Silver nanoparticles on VERO cells, the cell viability assay was done using 96-well plates. To determine the ability of silver nanoparticles in reduce the cytopathic effect of Influenza virus in VERO cells. Results: Silver nanoparticles was reduce of cytopathic effect of Influenza virus in Vero cells infected with Influenza virus (1:5 ) in the presence of silver nanoparticles at concentration 1 µM/ml. VERO cells infected with Influenza virus (1:5) in the presence of silver nanoparticles at concentration 10 µM/ml. and (1:5) in the presence of silver nanoparticles at concentration 25 µM/ml. Conclusion used silver nanoparticles to kill Influenza virus at concentration 25 µM/ml.

Keywords: Silver nanoparticles, Influenza virus, Vero cells


Introduction

The world population faces the problem of viral infection and the various life-threatening diseases caused by different viruses, such as the common cold, influenza, hepatitis, chickenpox, infectious mononucleosis, herpes keratitis, human immunodeficiency virus, and viral encephalitis. Much effort has been made to develop medicines and vaccines against a number of viruses (Tauxe, 2002; Domingo,2010)

Even though these coronaviruses cause infections throughout the year, spikes in infections occur during the winter and early spring months. As with other respiratory viruses, such as the influenza virus, the reasons for this are not entirely clear (figure 1).
The coronavirus is milder and less dangerous than the current human influenza virus that is affecting millions and killing many, this group of human coronaviruses typically infects all age groups; multiple reinfections are common throughout the lifespan of humans, so influenza pandemics remain a serious public health problem worldwide. Influenza virus-associated illnesses cause an estimated 200,000–500,000 hospital admissions and hundreds of thousands of deaths annually (Simonsen et al., 2000; Suwannakarn et al., 2010; Cox and Subbarao, 2000; Webby and Webster, 2003; Wang et al., 2010; Chu et al., 2009). Influenza virus infection is an important cause of respiratory diseases worldwide. Due to antigenic shifts and drifts of the virus, it is hardly managed with antiviral drugs (Carraro et al., 2007).

The emergence of variations in virus strains is one of the principal challenges facing prevention of infection with influenza virus. In response to rapid antigenic drift of influenza virus, extensive use of inactivated viral vaccines has been considered as the most effective approach (Bardiya and Bae, 2005). However, the production of vaccines may not be adequate to meet the increase in demand when an influenza pandemic occurs. Although the treatment of influenza produces rapid suppression of H1N1 influenza A virus infection in the short-term, this effect is not often sustainable due to the emergence of drug-resistant H1N1 strains. Therefore, it is important to develop new antiviral strategies to combat wild-type and mutant H1N1 influenza A virus infections. The development of new influenza antiviral drugs is urgent. Nanotechnology offers the ability to explore again the properties of materials by manipulating their sizes (Bonnemann and Richards, 2001).

The unique nature of nanoparticles has been exploited in the hope of developing novel diagnostic and antimicrobial agents (Percival et al., 2007; Shahverdiet al., 2007). Recently, metal nanoparticles have attracted increasing attentions for their important applications in a variety of areas, such as biosensors, labels for cells, biomolecules, and cancer treatment (Nam et al., 2003; Tkachenko et al., 2003; Hirsch et al., 2003). A notable application of nanoparticles lies in...
the antimicrobial properties of silver nanoparticles, which have received considerable attention because of their physicochemical properties. Silver nanoparticles have been shown to be a promising antimicrobial and are considered as an option for antiviral treatment (Sondi and Salopek, 2004; Samuel and Guggenbichler, 2004; Yang et al., 2007). Currently, studies on the interaction of silver nanoparticles with viruses are limited, this study, conducted for the first time in Iraq.

In the literature reviewed for this study, only six published papers on antiviral activities showed that silver nanoparticles can inhibit viral replication of viruses, such as HIV-1, hepatitis B virus, respiratory syncytial virus, herpes simplex virus type 1, and monkeypox virus (Sun et al., 2005; Elechiguerra et al., 2005; Lu et al., 2008; Sun et al., 2008; Pinto et al., 2009; Rogers et al., 2008). The studies found that silver nanoparticles can bind to HIV viral particles, thus inhibiting the virus from binding to host cells (Elechiguerra et al., 2005). In addition, silver nanoparticles can inhibit the DNA or RNA replication of the virus (Elechiguerra et al., 2005; Lu et al., 2008).

**Materials and Methods**

**Maintenance of cell cultures:** VERO cells were maintained in RPMI-1640 supplemented with 10% Fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were passaged using Trypsin-EDTA reseeded at 80% confluence twice a week, and incubated at 37 °C (Ali, M Jabir and Al-Shammari, 2019).

**MTT Assays:** To determine the cytotoxic effect of silver nanoparticles on VERO cells, the cell viability assay was done using 96-well plates. Cell lines were seeded at 1 × 10⁴ cells/well. After 24 hrs. or a confluent monolayer was achieved, cells were treated with silver nanoparticles at different concentration (1, 10, 25) µM/ml. Cell viability was measured after 72 hrs. of treatment by removing the medium, adding 28 µL of 2 mg/mL solution of MTT stain and incubating the cells for 2.5 h at 37 °C. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 130 µL of DMSO (Dimethyl Sulphoxide) followed by 37 °C incubation for 15 min with shaking (Ali et al., 2019). The absorbency was determined on a microplate reader at 492 nm; the assay was performed in triplicate. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation (Alsaedi et al., 2019):

\[
\text{Cytotoxicity} = \frac{A-B}{A} * 100
\]

Where A and B are the optical density of control and the optical density of test

**Silver nanoparticles inhibits cytopathic effect of Influenza virus:** To determine the ability of silver nanoparticles in reduce the cytopathic effect of Influenza virus in VERO cells. The Vero cells were seeded at 1 × 10⁴ cells/well. After 24 hrs. or a confluent monolayer was achieved, cells were infected with Influenza virus M.O.I. (1:5). In the presence and absence of silver nanoparticles at different concentration (1, 10, 25) µM/ml. Cell viability was measured after 96 hrs of infection with virus by removing the medium, adding 28 µL of 2 mg/mL solution of MTT and incubating the cells for 2.5 h at 37 °C. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 130 µL of DMSO (Dimethyl Sulphoxide) followed by 37 °C incubation for 15 min with shaking [2]. The absorbency was determined on a
microplate reader at 492 nm; the assay was performed in triplicate. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation:

\[
\text{Cytotoxicity} = \frac{A - B}{A} \times 100
\]

Where A and B are the optical density of control and the optical density of test.

For visualize the shape of cells under inverted microscope, 200 µL of cell suspensions were seeded in 96-well micro-titration plates at density \(1 \times 10^4\) cells mL\(^{-1}\) and infected influenza virus M.O.I. (1:5). In the presence and absence of silver nanoparticles at different concentration (1, 10, 25) µM/ml. Incubated for 48 hrs at 37°C. Then the medium removed. The plates were stained with 50 µL with Crystal violet and incubated at 37°C for 15 min, the stain was washed gently with tap water until the dye was removed. The cell observed under inverted microscope at 100x magnification microscope filed and photographed with digital camera (Kademetal., 2019).

**Statistical analysis:**

The obtained data were statically analyzed using an unpaired t-test with GraphPad Prism 6. The values were presented as the mean ± SEM of triplicate measurements (Jabir et al., 2018).

**Results and discussion:**

This study, conducted for the first time in Iraq, showed results three concentration of silver nanoparticles at different concentration (1, 10, 25) µM/ml, the 25 µM/ml as best concentration that kill all virus (figure -2)
Fig2: Cytotoxic effect of Silver nanoparticles in VERO cells.

The results in figure (3) showed Silver nanoparticles inhibits cytopathic effect of Influenza viruse in (25 µM/ml).
Fig3: Silver nanoparticles inhibits cytopathic effect of Influenza virus

So, results in figure (4) showed role of Silver nanoparticles in reduce of cytopathic effect of Influenza virus in Vero cells at three concentration, best kill of silver nanoparticles at concentration 25 µM/ml.
Fig 4: Role of Silver nanoparticles in reduce of cytopathic effect of Influenza virus in Vero cells. A, control-uninfected VERO cells. B, VERO cells infected with Influenza virus (1:5). C, VERO cells infected with Influenza virus (1:5) in the presence of silver nanoparticles at concentration 1 µM/ml. D, VERO cells infected with Influenza virus (1:5) in the presence of silver nanoparticles at concentration 10 µM/ml. E, VERO cells infected with Influenza virus (1:5) in the presence of silver nanoparticles at concentration 25 µM/ml.

Discussion:

Silver is widely used in industrial applications because of its metallic properties, such as conductivity, and in the medical field due to its antimicrobial effect (Alexander, 2009). Silver shows antibacterial activity against various organisms, and this effect is observed even at low concentrations (Ip, M *et al.*, 2006).
Silver nanoparticles can be used as antiviral agents against the H1N1 influenza virus (Xiang et al., 2011), human immune deficiency virus (HIV) (Elechiguerra et al., 2005), monkeypox virus (Rogers et al., 2008), or hepatitis B virus (Lu et al., 2008). Respiratory syncytial virus (Sun et al., 2008) but, the antiviral effects of silver nanomaterials against coronaviruses (CoVs) remain underdeveloped.

Studies on the antiviral action of Ag NPs are far behind those targeting microbicidal properties, and the mechanism of antiviral action is still not well understood. A viral infection is established when the nucleic acids of the virus are introduced into the host cell and then replicated. Ag NPs possibly act on the surface of the virus and physically inhibit the contact with host cells (Mehrbod et al., 2010; Devi and Joshi, 2012; Karmali, 2018).

References:

- Broadly protective monoclonal antibodies against H3 influenza viruses following sequential immunization with different hemagglutinins. J. PLoSPathog. 6, e1000796.


