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Full Length Article

Inhibitory effect of silver nanoparticles on bovine herpesvirus-1

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1. Introduction

Although remarkable improvement has been established from time to time in the area of antiviral therapy, medicines are unable to totally prevent all viral diseases. Hence, it is indispensable to develop antiviral agents which can act against a wide reach of viruses [1]. Nanotechnology field is one of the most active areas of research in contemporary material science. Every day, this field is emerging with new discoveries, causing an impact on the quality of human life [2]. The specific properties and characteristics of nanoparticles, such as size, shape, morphology and crystalline structure, make them fit for many applications. Silver nanoparticles (AgNPs) have been attracted by many researchers working in many fields as food technology, agriculture, medicine, and environmental technology [3]. In medicine, AgNPs are considered a novel therapeutic agent and widely studied as an antimicrobial agent [4]. It has a potential for the treatment of animal bacterial diseases [5]. Also, it attracts abundant attention due to the potential antiviral action, and is the topic of much research effort in the treatment of viral diseases [1] against monkey poxvirus [6], herpes simplex virus [7], human immune deficiency virus; HIV [8], poliovirus [9], and rift valley fever virus [10].

Virus infections pose significant global health challenges [3]. Some of the synthesized antiviral compounds are associated with other health hazards.

In Egypt, the utilization of nanotechnology in human and veterinary medicine has shown a great growth. Nanotechnology holds a big promise for animal health, veterinary medicine and many areas of animal production [11,12]. Nanoparticles proved as proficient therapeutic agent due to its outstanding physiochemical properties, characteristics and globally applicable physical mode of action [2]. There is a positive correlation between the high concentration of AgNPs and the inhibition of *Escherichia coli* (*E. Coli*) isolated from surface and ground water [13].

Infectious bovine rhinotracheitis/infectious bovine balanopothitis (IBR/IPV) is a highly contagious disease caused by BoHV-1 affecting cattle and buffaloes worldwide [14]. The disease causes severe reproductive disorders leading to significant economic losses to cattle industries [15].

In this study, *in vitro* determination of the inhibitory effect of Ag-NPs on viral replication (Bovine herpesvirus-1; BoHV-1 as a model) with an emphasis on the therapeutic, safe and cytotoxic concentrations was conducted.

2. Materials and methods

This research proposal was approved by the Ethical Research Committee of Animal Reproduction Research Institute, Code No. 181429 on 26-12-2017.

2.1. Silver nanoparticles preparation

Silver nanoparticles 755 μg/mL (0.07 mM as stock solution) were synthesized by the chemical reduction method as described before [16]. The solution was used to prepare two fold serial dilutions with varied concentration (755, 371, 188, 94, 47, 24, 12 and 6 μg/mL 2% MEM). The morphology and size of the synthesized silver nanoparticles were examined by high resolution transmission electron microscope type JEOL JEM- 1200 EX, operating at 120kv. UV–VIS spectrophotometer was used to detect the UV–visible spectrum of prepared nanoparticles.

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Particle size distribution of prepared nanoparticles was tested by Malvern-Zetasizer Nano (ZSP) – UK.

2.2. Virus and cell culture

The international reference (Colorado) strain cultured on BVD and Mycoplasma free Madin-Darby bovine kidney (MDBK) cell culture were used. The tissue culture infective dose 50 ($TCID_{50}$) was calculated according to Reed and Muench [17] method that was used to determine the viral titer. The virus suspension was used at $10^{5.8}$ $TCID_{50}$ to study the cellular viral effect and the anti-viral effect of Ag-NPs.

2.3. Cytotoxicity assay of Ag-NPs

The anti-viral effect of Ag-NPs on the viability of MDBK cells was measured using tetrazolium salt MTT {3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide} as colorimetric-based assay according to previous studies [18,19]. The infected and non-infected MDBK were treated with two fold serial dilutions of the Ag-NPs in 2% MEM (0.07 mM in solubilization solution as stock solution). Each dilution added in one column of 96 well tissue culture (TC) plate 25 µL/well. After 72 h, post incubation, cellular viability was measured by MTT and the optical density (OD) was read at wavelength 550 nm using an ELISA reader (Thermo Lab systems, Brussel, Belgium).

2.4. Anti-BoHV-1 effect of Ag-NPs

MDBK cells cultured in 96 well TC plate were infected with pre-incubated virus suspension mixed with Ag-NPs dilution as 25 µL/well for 2 h. The infected-, treated- and control MDBK were stained with neutral red (NR) stain. The NR stain was prepared as described by Repetto et al. [20] and used as 10% in the culture medium (0.33% as stock solution).

2.5. Data analysis

Data presented as mean ± SD and analyzed using a one-way analysis of variance (ANOVA) by using the SPSS 6.1.3 software package (SAS, Cary, NC, USA) and the difference between the means was measured by the test of least significant difference (LSD) at statistical significance $P < .05$.

3. Results

3.1. Properties of synthesized AgNPs

Transmission electron microscope TEM image of the prepared silver nanoparticles (Fig. 1) depicted spherical particles with size < 30 nm. Few particles were prismatic while others were cubic in shape. Owing to the high reactivity of the synthesized particles, they could agglomerate.

The prepared silver nanoparticles showed particle size distribution (Fig. 2). The particle size ranged between 15 and 50 nm with the average particle size of 20–25 nm. This result confirmed the results of transmission electron microscope.

Uv–visible spectrum of prepared silver nanoparticles is shown in (Fig. 3). As indicated in the spectrum, two absorption peaks at wavelength about 350 and 450 nm were observed. These peaks were attributed to the formation of spherical and deformed spherical silver particles.

3.2. Cellular safety and toxicity of Ag-NPs:

Ag-NPs were safe for the MDBK cell culture at a concentration of 24 µg/mL medium. The highest concentrations were cellular toxic with different degrees (Table 1).

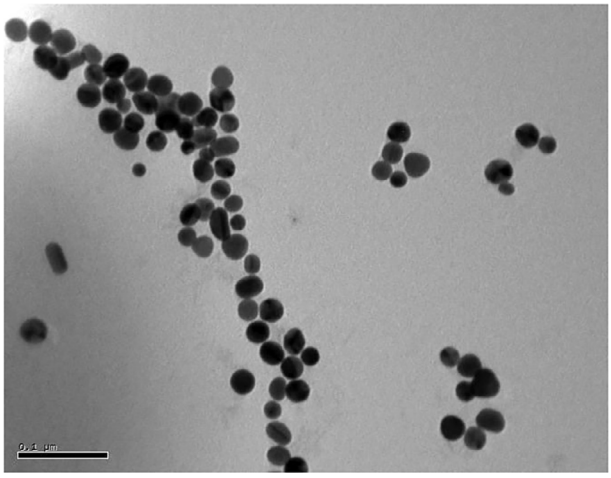

Fig. 1. Transmission electron microscope image of the synthesized silver nanoparticles.

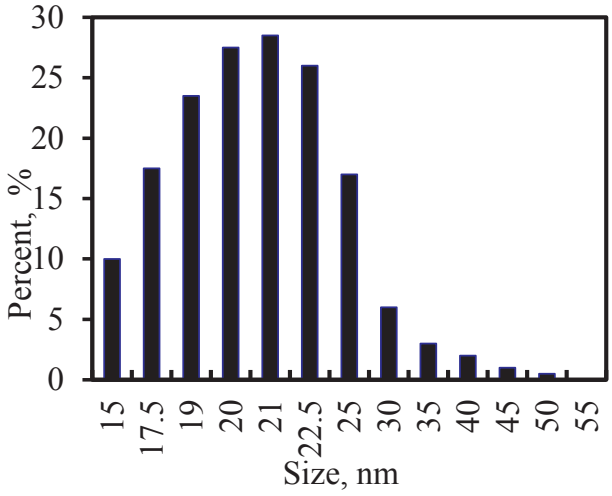

Fig. 2. Particle size distribution of the synthesized silver nanoparticles.

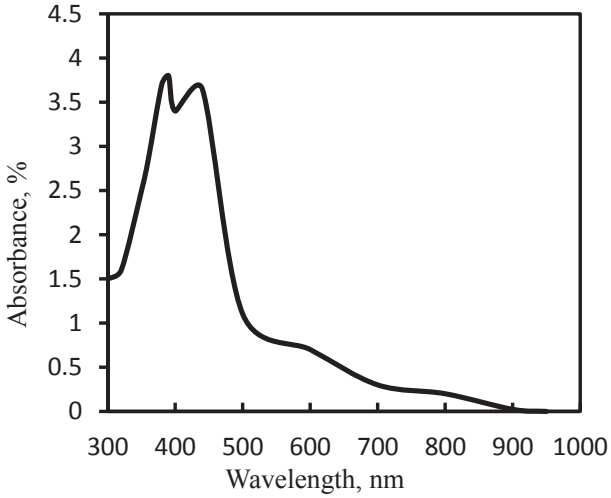

Fig. 3. Ultraviolet-visible spectrum of the synthesized silver nanoparticles.

3.3. The cytopathic effects of BoHV-1 in inoculated MDBK

BoHV-1 inoculated MDBK cells (Fig. 4a), were rounded, detached and appeared with apoptosis with chromatin condensation, cellular

Table 1
The cell viability results by MTT assay with different Ag-NPs concentrations (µg/mL).

Conc. µg/mL	Control	755	371	188	94	47	24	12	6
Mean ± SD	0.282 ± 0.041^{bc}	0.086 ± 0.013^{d}	0.240 ± 0.001^{c}	0.240 ± 0.009^{c}	0.257 ± 0.008^{c}	0.245 ± 0.008^{c}	0.274 ± 0.007^{bc}	0.270 ± 0.023^{bc}	0.321 ± 0.009^{ab}

Means with different superscript letters are different at least at $P < .05$.

blebbing, and fragmentation of DNA while in negative control (Fig. 4b), normal cells were observed.

3.4. Effect of Ag-NPs treated BoHV-1 inoculated cell culture

Ag-NPs treated BoHV-1 inoculated MDBK cells showed that the effective concentration was 24 µg/mL and this concentration interfered with the BoHV-1 replication with no CPEs detection (Fig. 5a and b).

4. Discussion

Silver nanoparticles catch the attention of scientific community and industrialist consideration due to their broad range of highly accepted utilization in biomedical fields and industry (especially its powerful antimicrobial effects, anti-inflammatory effect, and wound healing). However, the presenting studies using Ag-NPs for prevention and treatment of viral infections are limited, but still they can pave the way for other researchers to show their interest in dealing against viral infections [21].

The size and geometry of Ag-NPs are dependent on the synthetic methods (chemical, physical, photochemical and biological) [22]. Each method has its advantages and disadvantages, adopted for its synthesis. Moreover, Ag-NPs can be found in spherical, rod and triangular shape, or coated with polymer, biomolecules and sugars [23,12].

Viruses infect all forms of cellular life, eukaryotes and prokaryotes. Viruses reported as the main cause of disease and death in the world. *Bovine herpesvirus 1* (BoHV-1) is a member of the family *Herpesviridae*, subfamily *Alphaherpesvirinae* [24,25]. *BoHV-1* isolates were classified into subtypes 1 and 2, according to enzyme profiles [26]. BoHV-1 causes an economically important disease in cattle [27,28], which is sustained in herds by latency in nervous ganglia, the hallmark of herpesviruses, as well as in non-neural sites, lymph nodes and tonsils [29–32].

Infection of permissive cells with BoHV-1 accelerates cell death, in part due to apoptosis [33]. Viral gene expression is temporally arranged in three distinct phases: immediate early (IE), early (E), or late (L) [34]. During BoHV-1 productive infection, caspase 3 is activated relatively late, suggesting that apoptosis takes place [33,35,36]. In (Fig. 4a), MDBK cellular apoptosis were observed after 48 hr post infection (P.I) with BoHV-1. Many published studies assessed the effect of Ag-NPs on the HIV-1 virus [37,38]. The authors concluded that the interaction between Ag-NPs and virus is size dependent (small sized nanoparticles are more effective against these viruses) [37,39]. They further refined the idea that Ag-NPs get stuck to the sulphur present in the gp120 glycoprotein knobs that hinders the normal activities of the virus, therefore the normal functions of the virus is blocked. In this study, Ag-NPs can protect the MDBK cell culture from BoHV-1 infection at a concentration of 24 µg/mL medium (which is safe at the same time on MDBK). This is agree with the finding recorded by Hu et al [40] who reported that Ag-NPs could inhibit poliovirus and showed the nontoxic effect of the cell culture even at concentrations up to 100 ppm.

Also, Elechiguerra et al. [37] proposed that Ag-NPs can control the apoptosis of MDCK cells caused by H1N1 influenza A virus. Most reported articles recommended the same mechanism which states that Ag-NPs get stuck to the outer proteins of the virus sequentially inhibiting the normal function of the virus. As illustrated in the (Fig. 5a, b), the Ag-NPs at the recommended concentration inhibit the MDBK cellular apoptosis.

5. Conclusions

Ag-NPs could be used *In Vitro* safely at the recommended concentration (24 µg/mL) to protect the cellular culture against viral replications. Ag-NPs at nontoxic concentrations were capable of inhibiting BoHV-1 when administered prior to viral infection.

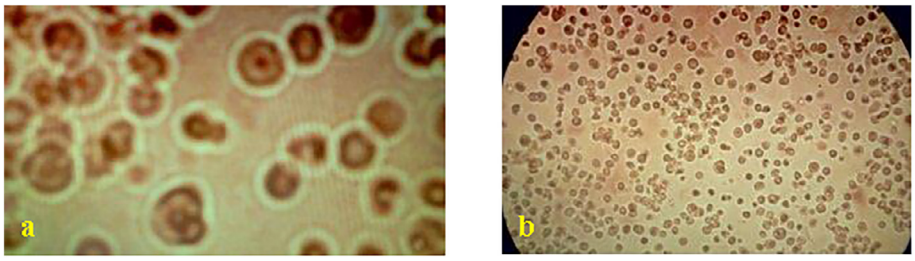

Fig. 4. (a) Bovine herpesvirus-1 inoculated Madin-Darby bovine kidney cells and stained with neutral red. (b) negative control cells.

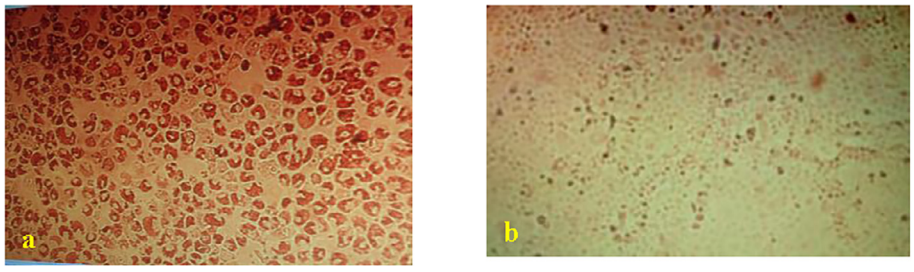

Fig. 5. (a) Silver nanoparticles treated bovine herpesvirus-1 inoculated Madin-Darby bovine kidney cells and (b) negative control cells.

6. Competing interests

The authors of the present work report that there is no conflict of interest in this work.

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