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Original Article

Investigation of the Inhibition Activity of Aluminium Oxide Nanoparticles for Herpes Simplex Type 1

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Abstract

Several studies have shown that Herpes simplex type 1 (HSV-1) is one of the viruses resistant to medications, so potential antiherpetic agents need to be evaluated. This study aimed to evaluate the impact of Aluminum Oxide Nanoparticles (Al_2O_3-NPs) on HSV-1 infection. Characterization of Al_2O_3-NPs was performed using field emission scanning electron microscopy (FESEM), X-ray diffraction (XRD), dynamic light scattering (DLS), and high-resolution transmission electron microscopy (HRTEM). The MTT test was used to investigate the toxicity action of Al_2O_3-NPs on viable cells. Quantitative Real-Time PCR (qRT-PCR)and TCID50 assays were used to achieve the antiherpetic performance Al_2O_3-NPs.Indirect immunofluorescence assay (IFA) was performed to determine the inhibitory impact of Al_2O_3-NPs on viral antigen expression, and acyclovir was utilized as a standard agent in all tests. HSV-1 subjected to Al_2O_3-NPs at the maximum non-toxic concentration (100 µg / mL) leads to a decrease of 0.1, 0.7, 1.8, and 2.5 log10 TCID50 in the infectious titer relative to virus control (P<0.0001). This concentration of Al_2O_3-NPs was correlated with 16.9%, 47.1 %, 61.2 %, 72.5 % and 74.6 % inhibition rates, calculated based on HSV-1 viral load compared to virus control. Our results have shown that Al_2O_3-NPs have a robust antiviral activity against HSV-1. This function demonstrates excellent potential for using Al_2O_3-NP in topical formulations for treating orolabial or genital herpetic lesions.

Keywords: Herpes simplex virus type 1, Aluminum oxide nanoparticles, Real-Time PCR, Antiviral activity, Indirect immunofluorescence assay

1. Introduction

Herpes simplex type 1 (HSV-1) is a highly infectious virus that is widespread and endemic worldwide. HSV-1 possesses double-stranded DNA, is subcategorized in herpesviridae and generally causes infections in the mouth, transmitted through direct contact with body fluids or individual infected lesions (1). HSV-1 has many clinical effects, such as orolabial abnormalities, peripheral nervous system disorders, encephalitis, facial abnormalities and corneal blindness (2). Both HSV-1 and HSV-2 are chronic diseases. Approximately 3.7 billion people under 50 (67%) are globally infected with HSV-1. Approximately 491 million people aged 15-49 (13%) are infected with HSV-2 (3). Antiviral drugs such as acyclovir, valacyclovir, and famciclovir are frequently the most drug treatments available for people with HSV infection. This antiviral drug is a deoxyguanosine analogue that generates its activity by inhibiting the DNA polymerase enzyme of HSV-1 (4). There is increasing evidence for the development of HSV-1-resistant strains, which have hindered the appropriate treatment of their infections (5). Besides, there are numerous studies on the adverse side effects

of these drugs, like abdominal pain, nausea, vomiting, neurotoxicity, skin rashes, and diarrhoea (6).

As a result, there is a rising need to develop and investigate new therapeutic agents for HSV-1 that have a different mechanism of action than traditional medications. NPs offer unique physical properties and have been developed to treat infectious diseases (7). These are primarily attributed to the particle size, which influences bioavailability and circulation time, and the wide area-by-volume ratio (increased solubility compared to larger particles). Such properties make ideal nanoparticulate candidates for investigating and enhancing therapeutic impacts (8, 9). Al_2O_3-NPs are a class of metal oxide NPs with varied biomedical applications due to their unique physicochemical and structural characteristics (10). By conjugating chosen ligands, proteins, antibodies, medicines, and enzymes to Al_2O_3-NPs, they may be tailored to the particular binding behaviour of selected target cells, increasing their targeted drug delivery capacity and therapeutic effectiveness at the disease site (11). This study aimed to develop methods for developing Al_2O_3NPs and evaluated them as antiviral therapy against pathogenic HSV-1 infection.

2. Materials and Methods

The Al_2O_3-NPs (powder form) were obtained from Merck Company (Germany; Catalogue Number: 108846). NPs were suspended in Dulbecco's Modified Eagle's medium (DMEM; Co., Ltd. (Shangdong, Yantai, China) to allow various concentrations, NPs suspension was subjected to sonication to reduce aggregation. Acyclovir agent was obtained from Sigma-Aldrich (Shanghai, China), dissolved in DMEM, and utilized in various concentrations as a typical agent for HSV-1 treatment.

2.1. Characterization of Nanoparticles

FESEM (Hitachi S-4160, Japan), HRTEM (Carl Zeiss AG-Zeiss EM900, Germany), DLS (Malvern Instruments Ltd., Malvern, UK), and XRD (SIEMENS-D5000) analysis were utilized to achieve morphological, dimensional, structural, and scale data on NPs.

2.2. Cell and Virus Culture

Fibroblast cells provided were obtained from the American Type Culture Collection (ATCC). DMEM with 10% fetal bovine serum and 100 g/mL streptomycin was used to culture fibroblast cells, 2 mM L-glutamine, 1 mM sodium pyruvate and 100 IU/mL penicillin (Sigma-Aldrich, USA). The cells were grown at 37°C in a humidified incubator with a 5% CO_2 environment. The vaccine and sera institute provided the HSV-1 stock (Baghdad, Iraq). HSV-1 was propagated in Fibroblast cells, titrated using the Reed and Muench formula for 50% tissue culture infectious dose (TCID50), and preserved in sterile cryovials at 80 °C.

2.3. MTT Assay

The methyl thiazolyl tetrazolium (MTT) test was used to assess the impact of Al_2O_3-NPs on fibroblast cell survival. In a 96-well flat-bottomed microtiter plate (Nalge Nunc (Naperville, IL)), fibroblast cells (1 105 cells/mL) were sown and cultured for 24 hours at 37 ° C. In triplicate, different Al_2O_3-NP concentrations (20 to 140 g / mL) were applied to the plate.5 µL of MTT powder (5 mg / mL) (Roche (Mannheim, Germany)) was applied to each well after 48 hours of incubation at 37 ° C, and the plate was incubated in the dark at 37 ° C for 3 hours. The MTT solution was then discarded, adding 50 µL of tidy dimethyl sulfoxide (Sigma-Aldrich, USA) to each well, and the plate was gently shaken for 10 minutes at ambient temperature. After that, the plate reading was performed at 550 nm in a microplate reader (Synergy4, Biotek Instruments, Winooski, VT, USA), and the cell viability ratio was measured for each dose compared with untreated control cells.

2.4. Determination of Antiviral Activity

In a humidified environment comprising 5% CO_2, confluent monolayers of fibroblast cells in a flat-bottomed 96-well microtiter plate were treated with 100 L of 100 TCID50/mL HSV-1 for 1 hour at 37°C. After removing the viral inocula, the monolayers were

rinsed and triplicated with papered PBS to eliminate any remaining viruses. The infected cells were cultured for 48 hours at 37°C in a humidified 5% CO_2 environment with 100 L of various noncytotoxic Al_2O_3-NPs. This experiment also contained a cell control (uninfected cells in DMEM) and a viral control (virus + DMEM). Acyclovir was also tested using this method. Following incubation, the cells were frozen and again thawed to liberate the cell-related virus bodies. Subsequently, the lysates from the wells were utilized in qRT-PCR and TCID50 experiments.

2.5. Quantitative RT-PCR

According to the manufacturer's instructions, the Genomic DNA isolation Mini Kit extracted HSV-1 DNA from the collected lysate (Qiagen, UK). The primers 5′-TGA GGC GCG ATT CTG GATGC-3′ and 5′-AAC GCG TCC TTG TTC TCG GC-3′were used to amplify the US3 region 127-bp fragment in a quantitative RT-PCR. The reaction conditions settings for RT-PCR were the same as in the previous work (12). RT-PCR was carried out by means of Rotor-Gene Q equipment (Qiagen, UK). A recombinant plasmid carrying a 127-bp DNA fragment of the HSV-1 US3 gene was utilized as a template. The segment was cloned onto the pGH plasmid vector and delivered as a lyophilized powder. The preparation of recombinant plasmid and cloning process was handled by Generay Biotech (Shanghai, China). To make a stock solution, 4 g of the template was weighed and dissolved in 40 L of dilution buffer to get a 100 ng/mL solution. After measuring the template DNA concentration by means of a NanoDrop spectrophotometer, the DNA template copy number in the standard stock was measured utilizing the Sequencing Center &URI Genomics software (Thermo Fisher Scientific, Grand Island, NY, USA). The serial dilution was performed for a stock solution ten times and used as a template to generate standard curves. The HSV-1 copy number in the unknown samples was determined using the standards.

2.6. Indirect Immunofluorescence Assay (IFA)

A 24-well tissue culture plate was utilized for fibroblast cell seeding and culturing in the wells using sterilized glass coverslips (Sigma-Aldrich, USA) (Qiagen, UK). After 85% confluency, the medium was withdrawn, and the cells were subjected to a humidified environment at 37 C with 5% CO_2 for 1 hour with 200 L of 100 TCID50/mL HSV-1 solution. The viral inocula were then withdrawn, and the plate was incubated at 37°C with the highest noncytotoxic concentration of ZnO-NPs. This experiment also contained a cell control (treated uninfected cells) and a viral control (untreated infected cells). The cells were fixed with cold acetone for 15 minutes after 14 hours of treatment, then treated with HSV-1 specific human antibody for 45 minutes at room temperature. After three washes with PBS, the cells were treated for 40 minutes at room temperature with goat anti-human IgG conjugated with fluorescein isothiocyanate (FITC) (Sigma-Aldrich, USA). The labelled cells were rinsed three times in PBS before being examined with an Olympus BH2-RFCA fluorescence microscope (Tokyo, Japan).

2.7. Statistical Analysis

GraphPad Prism software (Version 9.0) was applied to conduct statistical analyses, including one-way variance analysis (ANOVA) and Tukey's multiple comparison test. The error bars show the standard deviation, and the results represent the mean of three separate experiments. Statistical significance was determined by p-values less than 0.05.

3. Results

3.1. Characterization

DLS-determined Al_2O_3-NPs were found at 120.8±21.7 nm with 0.126±0.05 mV polydispersity index (PDI) (Figure 1A). As shown in figure 1B, the crystal structure of the Al_2O_3-NPs was studied with XRD. All nine diffraction peaks' position and relative \

strength corresponded well to the Normal Al_2O_3-NPs XRD sequence, comparable with the spectra previously mentioned (Figure 1B) (13). Morphology of Al_2O_3-NPs and their size Characterized by using FESEM and DLS techniques. FESEM images showed spherically distributed Al_2O_3-NPs with identical particle shape, size distribution, and 120 nm mean diameter (Figures 2A and 2B).

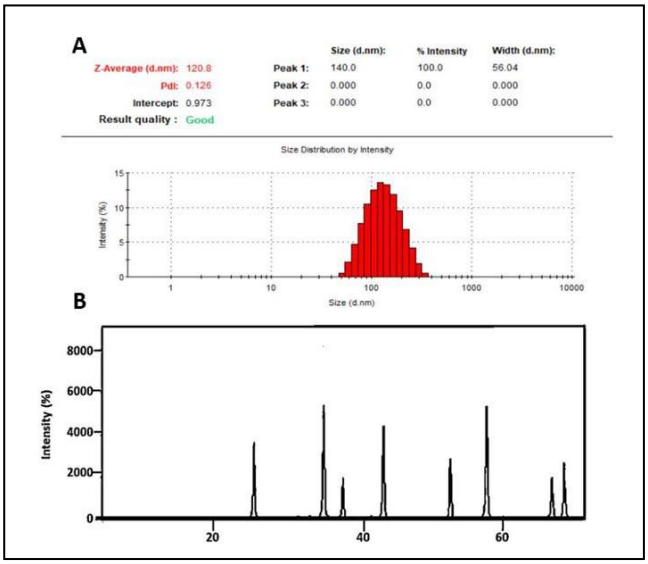

Figure 1. Size measurement images. The Al_2O_3-NPs size using dynamic light scattering (DLS) (**A**) and crystal structure of the Al_2O_3-NPs with XRD (**B**)

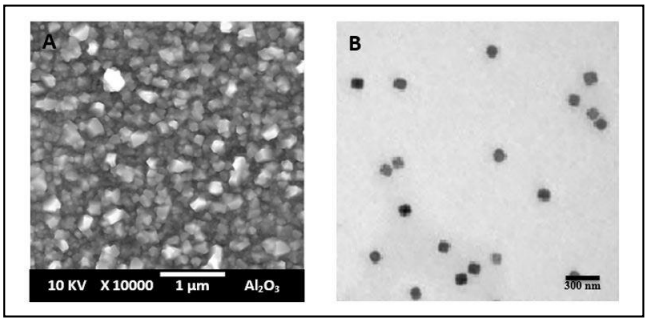

Figure 2. FESEM image of Al_2O_3-NPs (magnification 10 kx) (**A**); TEM image of Al_2O_3-NPs (**B**)

3.2. MTT Results

The MTT test was used to assess the cytotoxicity of Al_2O_3-NPs on fibroblast cells (See Figure 3). Figure 3 demonstrates the viability of fibroblast cells subjected to Al_2O_3-NPs at concentrations of 20, 40, 60, 80, 100, and 120 g/mL (P=0.0001). Vertical lines show three separate experiments' mean values. When the quantity of Al_2O_3-NPs was raised to 120 g/mL, the cell viability dropped to 48.32% compared to control cells. Antiviral tests were conducted using concentrations of Al_2O_3-NPs that had a cytotoxic impact of less than 10%.

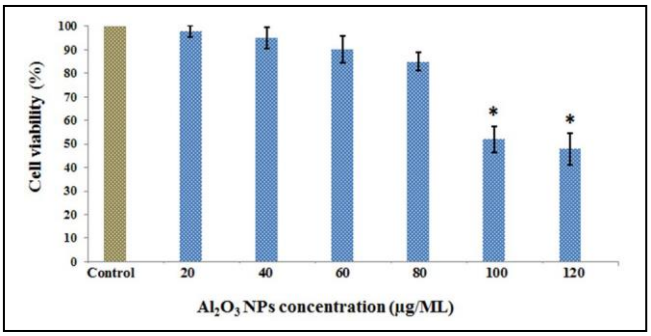

Figure 3. Cytotoxic study of Al_2O_3-NPs on fibroblast cells utilizing the MTT test

3.3. Cytopathic Effects (CPE)

In the presence of Al_2O_3-NPs, cytotoxic effects on fibroblast cells that HSV-1-infected are seen. The result indicated a reduced CPE caused by HSV-1 in Al_2O_3-NPs-untreated and treated HSV-1-infected Vero cells using an inverted microscope in a dose-dependent manner (Nikon, Japan). At a dosage of 100 g/ml, HSV-1-infected cells were treated with Al_2O_3-NPs. HSV-1 caused significant cytopathic effects in fibroblast cell monolayers 48 hours after viral injection, as shown in figure 4. These effects included cell rounding, refringence, and syncytia formation. In Al_2O_3-NPs-treated fibroblast cells infected with HSV-1, cytotoxic effects were decreased. The maximum utilized concentration (100 g/ml) of Al_2O_3-NP was linked with a roughly 10% cytotoxic impact on the fibroblast cells. As a result, the shape of specific cells has changed compared to the control, which is unrelated to HSV-1-stimulated cytopathic consequences (Figure 4).

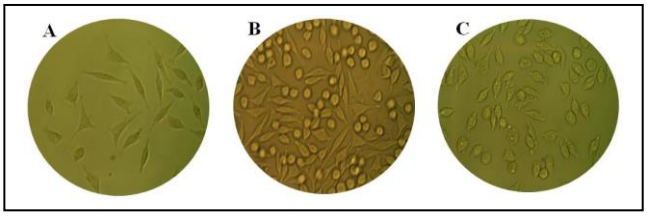

Figure 4. Prevention of cytopathic effects of HSV-1on fibroblast cells using Al_2O_3-NPs treatment (**A**) cell control (**B**) and virus control (**C**)

3.4. Evaluation of Antiviral Activity

The TCID50 test was used to evaluate the viability of fibroblast cells and the antiviral efficacy of Al_2O_3-NPs on the infected titer of HSV-1. The findings showed that Al_2O_3-NPs had a substantial inhibitory impact on HSV-1 activity at a concentration of 100 g/mL. When HSV-1 infected cells were exposed to 20, 40, 80, and 100 g/mL Al_2O_3-NPs, the infected titer of HSV-1 were reduced using 0.1, 0.7, 1.8, and 2.5 log10 TCID50, respectively, when in compared with viral control (*P*-values 0.0001) (Figure 5).

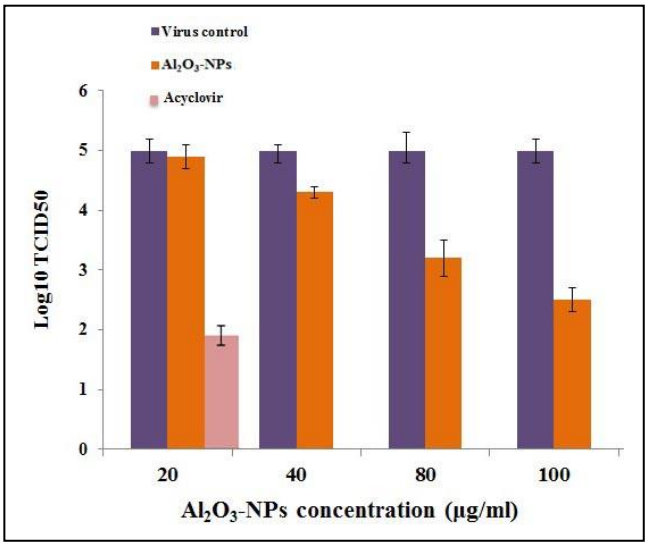

Figure 5. Comparison of antiviral effect of Al_2O_3-NPs and acyclovir on the infected titer of HSV-1 using the TCID50 test. When HSV-1 infected cells were exposed to 20, 40, 80, and 100 g/mL Al_2O_3-NPs, the infected titer of HSV-1 were reduced by 0.1, 0.7, 1.8, and 2.5 log10 TCID50, respectively, compared to viral control

3.5. Real-Time PCR

Real-time PCR was used to study the activity of Al_2O_3-NPs on HSV-1 viral load, which was established on the multiplication of a 127-bp segment from an exceedingly preserved location of the HSV-1 US3 gene by means of SYBR Green qRT-PCR Master Mix. The activity of Al_2O_3-NPs on the HSV-1 virus, as specified using real-time PCR, is shown in figure 6. The inhibition rates of Al_2O_3-NPs at different concentrations (20, 40, 60, 80, and 100 g/mL) were 16.9%, 47.1, 61.2, 72.5, and 74.6%, respectively, depending on the HSV-1 viral load (Figure 6). At a 40 g/mL dose, acyclovir showed full suppression of the HSV-1 infectious titer and a 100% inhibition rate (*P*=0.0001).

3.6. IFA Results

This experiment assesses the effect of Al_2O_3-NPs treatment on HSV-1 antigen expression in fibroblast cells using an immunofluorescence test (IFA). In an IFA research, Al_2O_3-NPs were found to have inhibitory effects on the expression of the HSV-1 antigen on the fibroblast cell surface. According to virus control, cell control, and HSV-1 infected cells; the cells were treated with 100 g/mL Al_2O_3-NPs. When HSV-1 infected cells were treated with Al_2O_3-NPs, the intensity of fluorescence signals was noticeably reduced compared to virus control, confirming that Al_2O_3-NPs had a potent antiviral impact on HSV-1 antigen expression (Figure 7).

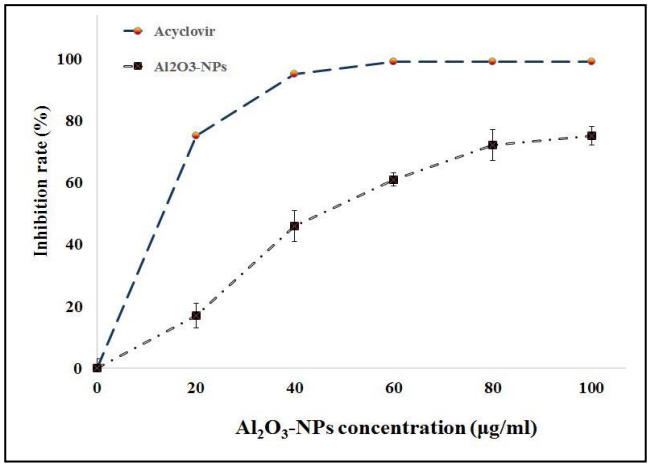

Figure 6. Real-time PCR analysis of the effect of Al_2O_3-NPs on HSV-1 viral load. The inhibition rates of Al_2O_3-NPs at doses of 20, 40, 60, 80, and 100 g/mL were 16.9, 47.1, 61.2, 72.5, and 74.6%, respectively, depending on the HSV-1 viral load

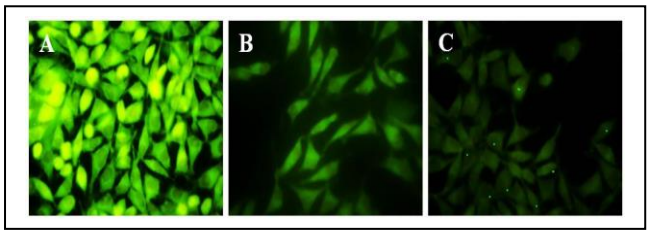

Figure 7. The impact of Al_2O_3-NPs on the HSV-1 antigens expression of fibroblast cells was assessed using an immunofluorescence test (IFA). HSV-1 infected cells were treated with 100 g/mL Al_2O_3-NPs. (**A**) Virus control, (**B**) Cell control, (**C**) HSV-1 infected cells treated with 100 g/mL Al_2O_3-NPs. When HSV-1 infected cells were treated with Al_2O_3-NPs, the intensity of fluorescence signals was substantially reduced compared to the virus control, suggesting that Al_2O_3-NPs had a robust antiviral effect on HSV-1 antigen expression. The green spots in figure 7 (**C**) represented the expression level of viral antigens in various cell sections and were discoloured with goat anti-human IgG coupled with FITC

4. Discussion

NPs have been widely researched for use as medication carriers and antibacterial agents in various areas (14). Aside from that, NPs have broad antiviral efficacy due to their multitargeting chemical processes. Viruses such as the human immunodeficiency virus, hepatitis B virus, and herpes simplex virus are resistant to silver NPs (15). Aluminium is an all-encompassing metal element especially suited for various applications in biomedicine (16). In recent years, NPs of aluminium oxide and their compounds have been identified as active agents for various pathogens (17). Due to minimal studies in this regard, it is important to study the inhibitory capacity of Al_2O_3-NPs for viral infection, especially against HSV-1. Based on the findings, Al_2O_3-NPs may be a new antiviral component for HCV-1 treatment. Within the culture system of fibroblast cells, the Al_2O_3-NPs were shown to suppress HSV-1 infection substantially. In our research, many laboratory techniques, including TCID50, Real-Time PCR, and IFA Assays, verified the suppression of viral infection. We aimed to evaluate the antiviral effectiveness of Al_2O_3-NPs injected into the cell following HSV-1 adsorption. When HSV-1 infects a cell, it transports the nucleocapsid from the cytosol to the nucleus via nuclear membrane pores. All processes of viruses, such as capsid development, gene replication, expression, and DNA packing, occur in the nucleus of infected cells (18). In one or more of those phases of viral replication, Al_2O_3-NPs may be interfering factors (19). Aluminium ions released from Al_2O_3-NPs were found to affect various targets directly and result in ROS development, resulting in DNA denaturation and cell integrity damage (20). We hypothesize that these action mechanisms can also help against viral infections.

In summary, according to our findings, Al_2O_3-NPs have considerable antiviral efficacy against HSV-1. The antiviral behaviour of Al_2O_3-NPs is thought to be explained by various mechanisms, such as the production of reactive oxygen species by free Al ions from NPs, which may contribute to HSV-1 inactivation through viral protein oxidation or viral genome destruction.

Authors' Contribution

Author contributions Conceptualization: S. A. M., A. K. A, and H. A., Methodology: S. A. M, A. K. A, Validation: S. A. M., A. K. A, B. B, Formal Analysis: B. B., Investigation: S. A. M., A. K. A., Resources: S. A. M., A. K. A, H., B. B., Writing–Original Draft Preparation: S. A. M., A. K. A., Writing – Review and Editing: S. A. M., Supervision: S. A. M., A. K. A.

Conflict of Interest

The authors declare that they have no conflict of interest.

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References

1. Zhang J, Liu H, Wei B. Immune response of T cells during herpes simplex virus type 1 (HSV-1) infection. J Zhejiang Univ Sci B. 2017;18(4):277-88.
2. Duarte LF, Farías MA, Álvarez DM, Bueno SM, Riedel CA, González PA. Herpes simplex virus type 1 infection of the central nervous system: insights into

proposed interrelationships with neurodegenerative disorders. Front Cell Neurosci. 2019;13:46.

3. WHO. Herpes simplex virus. 2022 [Available from: https://www.who.int/news-room/fact-sheets/detail/herpes-simplex-virus.
4. Trigilio J, Antoine TE, Paulowicz I, Mishra YK, Adelung R, Shukla D. Tin oxide nanowires suppress herpes simplex virus-1 entry and cell-to-cell membrane fusion. PLoS One. 2012;7(10): 48147.
5. Kongyingyoes B, Priengprom T, Pientong C, Aromdee C, Suebsasana S, Ekalaksananan T. 3, 19-isopropylideneandrographolide suppresses early gene expression of drug-resistant and wild type herpes simplex viruses. Antivir Res. 2016;132:281-6.
6. Kakiuchi S, Tsuji M, Nishimura H, Yoshikawa T, Wang L, Takayama-Ito M, et al. Association of the emergence of acyclovir-resistant herpes simplex virus type 1 with prognosis in hematopoietic stem cell transplantation patients. J Infect Dis. 2017;215(6):865-73.
7. Albukhaty S, Al-Musawi S, Abdul Mahdi S, Sulaiman GM, Alwahibi MS, Dewir YH, et al. Investigation of dextran-coated superparamagnetic nanoparticles for targeted vinblastine controlled release, delivery, apoptosis induction, and gene expression in pancreatic cancer cells. Molecules. 2020;25(20):4721.
8. Al-Musawi S, Albukhaty S, Al-Karagoly H, Sulaiman GM, Jabir MS, Naderi-Manesh H. Dextran-coated superparamagnetic nanoparticles modified with folate for targeted drug delivery of camptothecin. Adv Nat Sci Nanosci Nanotechnol. 2020;11(4):045009.
9. Haider AJ, Al-Kinani MA, Al-Musawi S, editors. Preparation and Characterization of Gold Coated Super Paramagnetic Iron Nanoparticle Using Pulsed Laser Ablation in Liquid Method. Key Engineering Materials; 2021: Trans Tech Publ.
10. Xiang D-x, Chen Q, Pang L, Zheng C-l. Inhibitory effects of silver nanoparticles on H1N1 influenza A virus in vitro. J Virol Methods. 2011;178(1-2):137-42.
11. Lu L, Sun RW-Y, Chen R, Hui C-K, Ho C-M, Luk JM, et al. Silver nanoparticles inhibit hepatitis B virus replication. Antivir Ther. 2008;13(2):253-62.
12. Mahdi SA, Kadhim AA, Albukhaty S, Nikzad S, Haider AJ, Ibraheem S, et al. Gene expression and apoptosis response in hepatocellular carcinoma cells induced by biocompatible polymer/magnetic nanoparticles containing 5-fluorouracil. Electron J Biotechnol. 2021;52:21-9.
13. Sezer N, Evis Z, Kayhan SM, Tahmasebifar A, Koç M. Review of magnesium-based biomaterials and their applications. Journal of magnesium and alloys. 2018;6(1):23-43.
14. Al-Musawi S, Ibraheem S, Abdul Mahdi S, Albukhaty S, Haider AJ, Kadhim AA, et al. Smart nanoformulation based on polymeric magnetic nanoparticles and vincristine drug: a novel therapy for apoptotic gene expression in tumors. Life. 2021;11(1):71.
15. Maruzuru Y, Shindo K, Liu Z, Oyama M, Kozuka-Hata H, Arii J, et al. Role of herpes simplex virus 1 immediate early protein ICP22 in viral nuclear egress. Virol J. 2014;88(13):7445-54.
16. Dziuba N, Ferguson MR, O'Brien WA, Sanchez A, Prussia AJ, McDonald NJ, et al. Identification of cellular proteins required for replication of human immunodeficiency virus type 1. AIDS Res Hum Retrovir. 2012;28(10):1329-39.
17. Zhang Q, Wang H, Ge C, Duncan J, He K, Adeosun SO, et al. Alumina at 50 and 13 nm nanoparticle sizes have potential genotoxicity. J Appl Toxicol. 2017;37(9):1053-64.
18. Huet A, Huffman JB, Conway JF, Homa FL. Role of the herpes simplex virus CVSC proteins at the capsid portal vertex. Virol J. 2020;94(24): 01534-20.
19. Ibáñez FJ, Farías MA, Gonzalez-Troncoso MP, Corrales N, Duarte LF, Retamal-Díaz A, et al. Experimental dissection of the lytic replication cycles of herpes simplex viruses in vitro. Front. Microbiol. 2018:2406.
20. Carmody CM, Goddard JM, Nugen SR. Bacteriophage Capsid Modification by Genetic and Chemical Methods. Bioconjug Chem. 2021;32(3):466-81.