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In vitro Virucidal Activity of Silver Nanoparticles against H1N1 Influenza A Virus and Herpes Simplex Virus-1

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Abstract: Silver nanoparticles (AgNPs) have provided a broad spectrum of antiviral activity against some types of enveloped and non-enveloped viruses. Silver nanoparticles are currently the most widely commercialized nanomaterials. In recent times, viral infections are emerging as one of the most common diseases with high mortality in the human population. The present study investigates the antiviral efficacy of the silver nanoparticles synthesized by the green method against HSV-1 and H1N1 influenza viruses. Characterization studies revealed that the silver nanoparticles showed an average particle size of 47 nm with a surface plasmon resonance peak at 426 nm. Silver nanoparticles exhibited 50% cytotoxicity in Vero cells at 197 µg/mL concentration, and 50% Inhibitory concentration (IC_{50}) against HSV-1 was observed at 19.6 µg/mL. Silver nanoparticles demonstrated > 1 log reduction in H1N1 influenza A virus at 17 µg/mL. These findings indicate that silver nanoparticles possess excellent antiviral activity, which can be suitably used in various formulations to eradicate the spread of viral infections.

Keywords: cytotoxicity; HSV-1; H1N1; nanoparticles; antiviral.

1. Introduction

Infectious diseases caused by viruses are a serious threat to the human population. Many types of viral infections exist, and the mortality rate varies depending on the virulence factors. Herpes simplex virus-1 (HSV-1) is an enveloped and double-stranded DNA virus that belongs to the Herpesviridae family [1]. Transmission of HSV-1 occurs through oral-to-oral or oral-genital contact. HSV-1 infections cause mollaret's meningitis, encephalitis, neonatal herpes, and blindness in humans [2]. Approximately 1.5 million cases yearly suffer from blindness due to HSV-1 infections in developed countries, and 40,000 new cases worldwide [3].

Influenza A virus is a respiratory pathogen in humans and causes a high mortality rate year [4]. Influenza A virus is further classified into various subtypes depending on the surface glycoproteins present viz. H1N1, H3N2, H5N1, and H7N9 [5]. In 2015, the Indian swine flu outbreak due to H1N1virus caused a 6% fatality in Rajasthan's middle-aged and younger population [6].

Antiviral drugs, namely acyclovir, valaciclovir, and ganciclovir, are used to treat HSV infections [7]. Influenza virus infections are treated by drugs such as favipiravir, oseltamivir, and zanamivir [8]. Although these drugs are known to inhibit the replication and proliferation of viruses, there exists only poor to negligible activity toward the mutated virus [9]. To address

this, there is an urgency to develop antiviral agents which act against the virus at multiple steps of viral replication.

With the advent of green nanotechnology, biomedical science is revolutionizing. The green chemistry approach for the synthesis of nanoparticles has gained more attention. Amongst, silver nanoparticles are widely applied as antimicrobial agents against bacteria [10], fungi [11], and viruses [12]. Recently, silver nanoparticles used have been as antiviral agents such as hepatitis B virus [13], HIV [14], Chikungunya virus [15], HSV-2 [16], Influenza virus (H3N2) [17], Bovine herpesvirus-1 [18]. Owing to the superior advantages, the nanoparticles developed by the green approach are considered an ideal test candidate for antiviral studies. The present study investigates the antiviral activity of silver nanoparticles developed by green nanotechnology. The antiviral efficacy of silver nanoparticles against herpes simplex type 1 (HSV-1) and influenza A viruses (H1N1) is evaluated.

2. Materials and Methods

2.1. Synthesis and characterization of silver nanoparticles.

Briefly, silver nitrate was mixed with amino acid conjugate with Tris (Hydroxymethyl) phosphine, and the mixture was transferred to a water bath sonicator and kept for 24 hours at 80°C [19]. The silver ion to the silver nanoparticles' formation was recorded using a UV-Vis spectrophotometer (SHIMADZU UV 2600). The surface morphology and elemental analysis of the silver nanoparticles were analyzed using High-Resolution Scanning Electron Microscopy (HR-SEM) and Energy Dispersive X-ray analysis (EDAX) (Oxford Instruments, X-Max 80mm^2). The morphology and size of silver nanoparticles were analyzed by Transmission Electron Microscopy (TEM; JEOL 1400, LTE, Tokyo, Japan).

2.2. Cell culture.

Vero cell lines were grown in Dulbecco's Modified Eagle Medium supplemented with fetal bovine serum (10 %) and then incubated at 5% CO_2 and 37°C until the cells were confluent around 70-80%. After 24 h, cell culture was harvested using trypsin, and approximately 2.0 x 10^5 cells/ml were seeded using a medium. MDCK cells (Madin-Darby Canine Kidney cells) were seeded using minimum essential medium (MEM) added with 10% BSA, 5% streptomycin, 0.025% trypsin, and glucose solution, and cells were incubated for 24h.

2.3. In vitro cytotoxicity assay.

The cytotoxicity of silver nanoparticles was studied in Vero cells using an MTT assay [20]. Approximately 1.0 x 10^5 cells were seeded into 96 well using 10% of FBS with DMEM medium and then incubated at 5% CO_2 and 37 °C for 24 h. After 24 h the medium was discarded, and different concentrations of silver nanoparticles (50, 100, 150, 200, 250, 300 µg/ml) were added to the fresh growth medium. Cells without treatment were considered as control, and then cells were incubated for 24 h. After 24 h treatment 50 µl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT - 5mg/ml) was added to each well. After 4h incubation, 100 µl of Dimethyl sulfoxide (DMSO) was added to each well to solubilize the formed formazan crystals, and the plate read at 570nm using a microplate reader.

$$\%\ of\ cells\ viability = \frac{OD\ value\ of\ treated\ group}{OD\ value\ of\ treated\ group}\ x100$$

2.4. In vitro anti-HSV-1 activity.

The virucidal activity of silver nanoparticles against HSV-1 virus was studied using the co-treatment method [21]. After 24 h, Vero cells were infected by HSV-1 at the dose of 100 TCID50 for 2 h. After that, cells were washed with PBS and replenished with 100 µl of medium and treated with different concentrations of the silver nanoparticles (50, 25, 12.5, 6.25 µg/ml). Control cells were treated with an infective dose of HSV-1 virus and incubated at 37°C and 5% CO_2. After 72 h treatment, 50 µl of MTT solution (5 mg/ml) was added to each well for 4 h at 37°C. Then, 100 µl of DMSO was added to dissolve the formazan crystals. After 10, min plate was read using an automated microplate reader at 570 nm. The percentage of cell protection/virus inhibition can be calculated as:

$$\frac{\text{(Mean OD of the control group – Mean OD of the treated group)} \times 100}{\text{Mean OD of the control group}}$$

2.5. In vitro Anti-influenza H1N1 virus activity.

The virucidal activity of silver nanoparticles against H1N1 virus was studied using the pre-treatment method [12]. Silver nanoparticles (17 µg/mL) were incubated with an equal volume of Influenza H1N1 virus, and the mixture was made using a cell culture medium. This mixture of virus and silver nanoparticles was kept at 37 °C for 1 h. After the incubation period mixture was filtered using a resin column (Microspin S-400HR). The collected eluate was used to infect MDCK cells. The infected MDCK cells were grown in MEM with FBS. The cells were maintained at 5% CO_2 at 37ºC for 6-7 days and observed for the presence of cytopathic effect (CPE).

3. Results and Discussion

3.1. Characterization of silver nanoparticles.

The silver nanoparticles showed the characteristic surface resonance peak of silver nanoparticles at 426 nm (Fig. 1a), which confirms the formation of the nanoparticles. Silver nanoparticles were well separated, and no visible aggregation was observed in SEM micrographs. (Fig. 1b). EDAX spectrum showed strong signals for the elemental silver of silver nanoparticles (Fig. 1c). TEM images confirmed the silver nanoparticles were spherical with an average size from 21 to 66 nm (Fig.2). Moreover, the particles were well separated. No aggregations were seen, and the same was consistent with SEM results.

3.2. Cytotoxicity assay.

As a first step, the biocompatibility of silver nanoparticles was assessed. Investigation of the *in vitro* cytotoxicity of silver nanoparticles (50, 100, 150, 200, 250, 300 µg/mL) on Vero cells revealed that a 50% cytotoxic dose (CC_{50}) was observed at the concentration of 197

µg/mL (Fig. 3A). Morphological observation of treated Vero cells clearly showed cytoplasmic condensation and cell shrinkage when compared with control (Fig.3B&C).

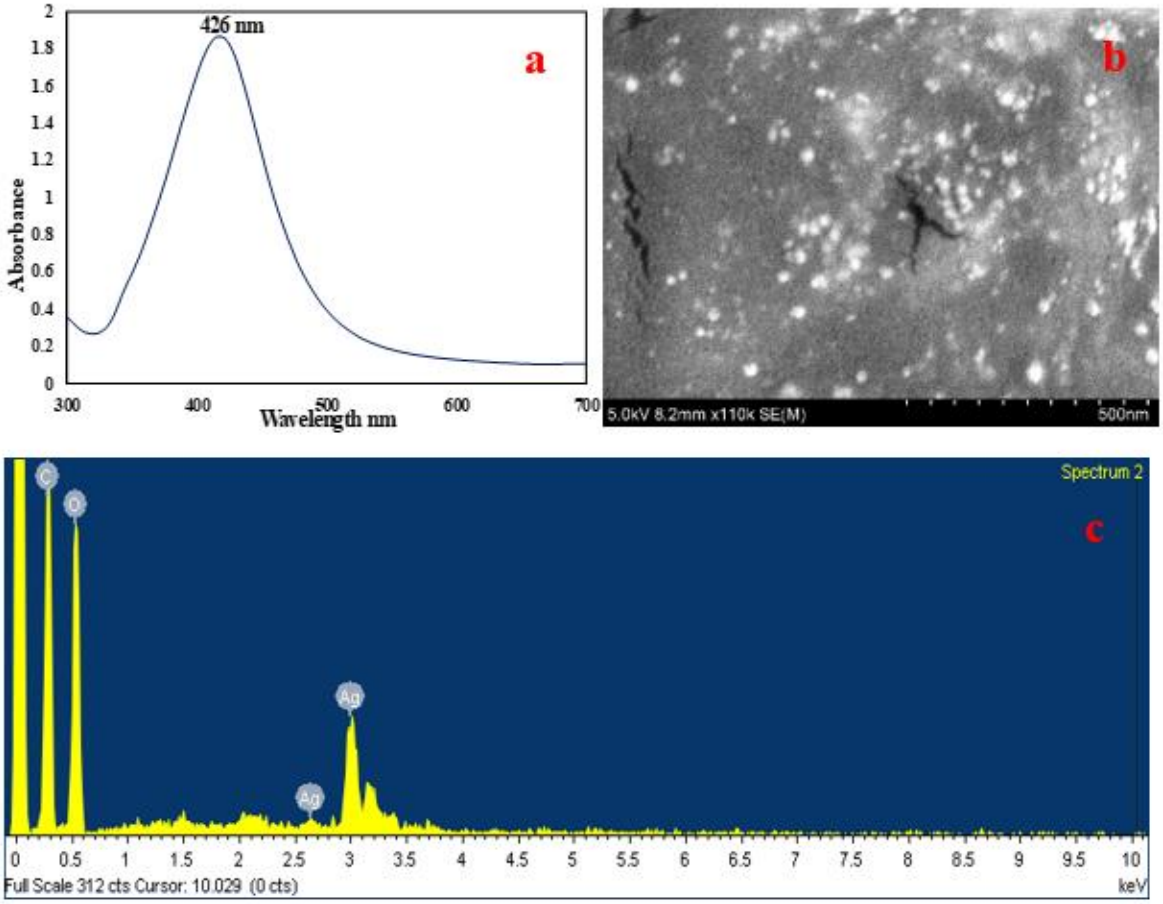

Figure 1. a) UV-Visible spectrum of Silver nanoparticles b) SEM image of silver nanoparticles, c) EDAX spectrum of silver nanoparticles.

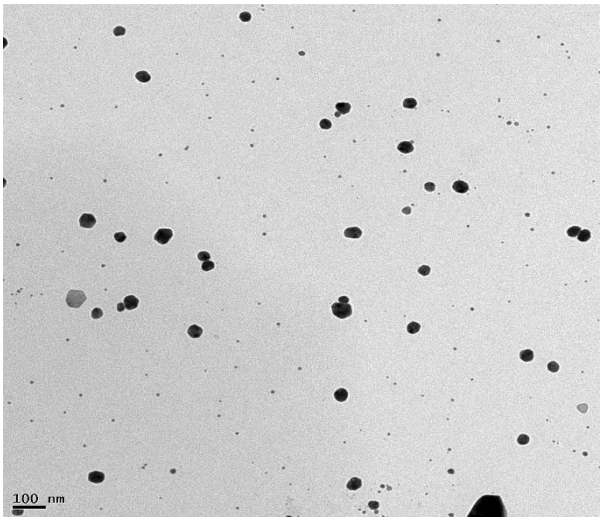

Figure 2. TEM image of silver nanoparticles.

3.3. Antiviral activity HSV-1 of silver nanoparticles.

The antiviral effect of silver nanoparticles against HSV-1 was determined by co-treatment of the virus with different concentrations of silver nanoparticles (6.25, 12.5, 25, 50 µg/mL). About 50% of viral inhibition was observed at 19.6 µg/mL (Fig.4A-C). Silver

nanoparticles showed anti-HSV activity of 92.8 % at 100 µg/ml. As the concentration of the test sample increases, the percent of age viral titer decreases. These results confirmed that silver nanoparticles display potent dose-dependent antiviral activity against HSV-1. In a report by Orlowski *et al*. [22], tannic acid-capped Ag NPs showed to inhibit the HSV-2 virus where the biological affinity with viral glycoproteins was increased, which aids in blocking post-infection, attachment, and penetration stage during virus replication. *Sargassum wightii* seaweed used to synthesize silver nanoparticles effectively reduced the 70% cytopathic effect of both HSV-1 and HSV-2 at 2.5 µL [23]. HSV-2 replication was inhibited at concentrations of 100 µg/mL and limited toxicity to cells [16].

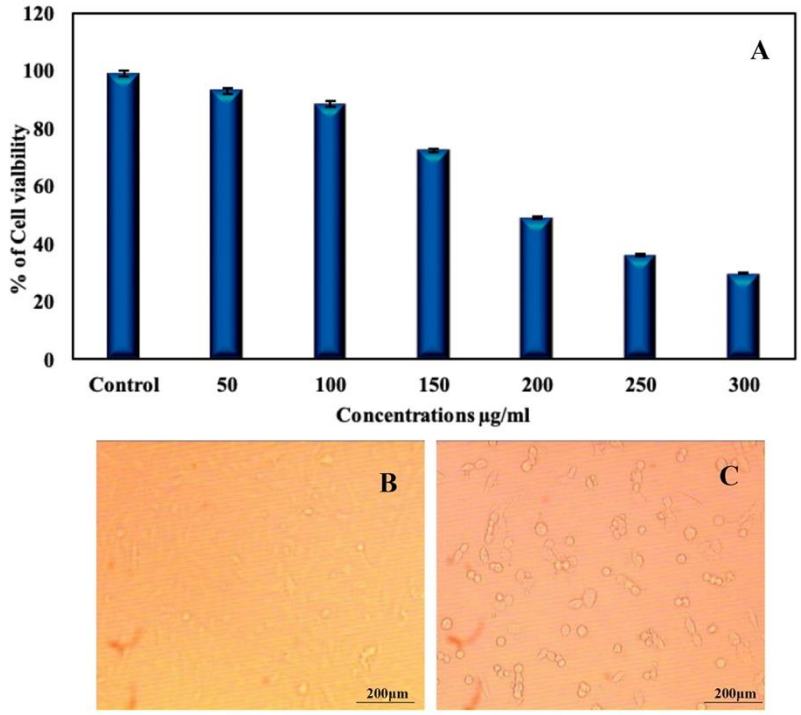

Figure 3. A. Cytotoxic effect of silver nanoparticles on Vero cells, B. Control Vero cells, C. Silver nanoparticles treated cells.

3.4. Anti-Influenza activity of silver nanoparticles.

MDCK cells were used for the proliferation of H1N1 virus. Interestingly, pre-treatment of the virus with 17 µg/mL of silver nanoparticles showed to inhibit virus propagation by a 1.2 log reduction from its initial titer. In a report by Xiang *et al.* [12], silver nanoparticles are shown to interact with the H1N1 virus. The possibility of viral inhibition occurs by different steps as follows, i) Ag NPs bind to the surface protein of the virus and inhibit the virus interaction to the normal cells (ii) Ag NPs bind to the genetic material of the virus (DNA or RNA) and inhibit the replication or propagation of the virus. Jeremiah *et al.* [24] reported that a Luciferase-based pseudovirus entry assay revealed that PVP-$AgNP_{10}$ potently inhibited viral entry and effectively inhibited extracellular SARS-CoV-2 to protect the target cells from infection. AgNPs at the dose of 50 µg/mL most effectively decreased the respiratory syncytial virus RSV replication by 79% in A549 cells and 78% in HEp-2 cells. AgNPs have the potential to block the entry of RSV by binding surface glycoproteins and/or inhibit the spread of RSV [25].

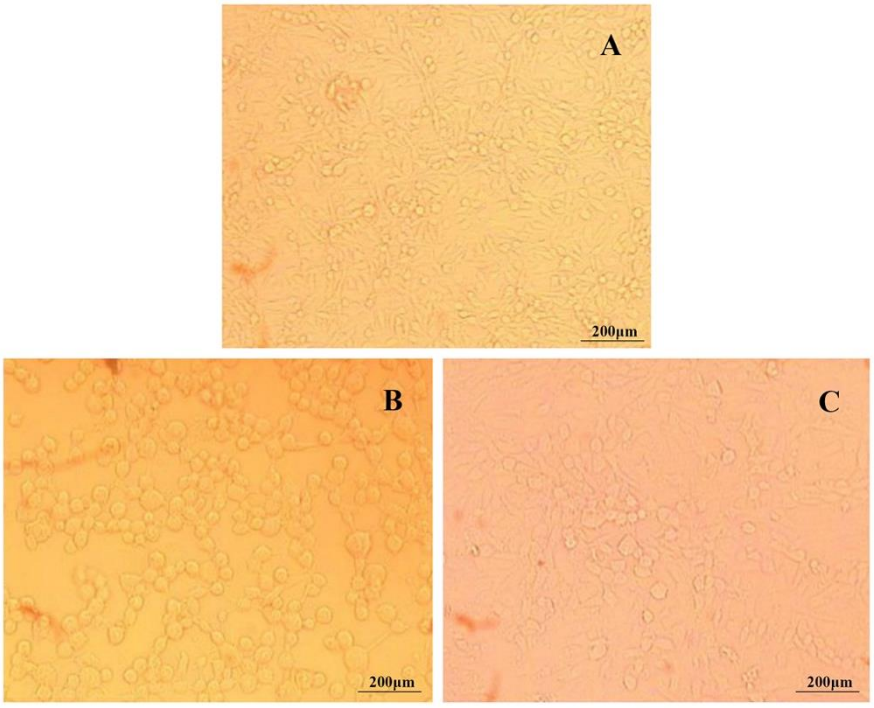

Figure 4. A. Vero cells, B. Vero cells infected with HSV-1, C. HSV-1 infected cells treated with silver nanoparticles.

4. Conclusions

This study presents the virucidal activity of the Silver Nanoparticles prepared by the biogenic approach against HSV-1 and H1N1 influenza A virus. Interestingly, silver nanoparticles displayed dose-dependent anti-HSV-1 activity with IC_{50} of 19.6 µg/mL. Moreover, more than a 90% reduction in H1N1 virus titer was observed with silver nanoparticles at 17 µg/mL. These results confirmed that silver nanoparticles display potential antiviral activity against HSV-1 and H1N1 influenza A virus. Considering this, silver nanoparticles, as an antiviral agent, can be readily used to prepare various formulations ranging from sanitizers to disinfectants, where the antiviral properties of silver nanoparticles could be of great use.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Madavaraju, K.; Koganti, R.; Volety, I.; Yadavalli, T.; Shukla, D. Herpes simplex virus cell entry mechanisms: An update. *Front. Cell. Infect. Microbiol.* **2021,** *10*, https://doi.org/10.3389/fcimb.2020.617578.
2. Koujah, L.; Suryawanshi, R.K.; Shukla, D. Pathological processes activated by herpes simplex virus-1 (HSV-1) infection in the cornea. *Cell. Mol. Life Sci.* **2019,** *76*, 405-419, https://doi.org/10.1007/s00018-018-2938-1.
3. Lobo, A.M.; Agelidis, A.M.; Shukla, D. Pathogenesis of herpes simplex keratitis: The host cell response and ocular surface sequelae to infection and inflammation. *The ocular surface* **2019,** *17*, 40-49, https://doi.org/10.1016/j.jtos.2018.10.002.
4. Macias, A.E.; McElhaney, J.E.; Chaves, S.S.; Nealon, J.; Nunes, M.C.; Samson, S.I.; Seet, B.T.; Weinke, T.; Yu, H. The disease burden of influenza beyond respiratory illness. *Vaccine* **2021**, *39*, A6-A14, https://doi.org/10.1016/j.vaccine.2020.09.048.
5. Park, J.E.; Ryu, Y. Transmissibility and severity of influenza virus by subtype. *Infection Genetics and Evolution* **2018**, *65*, 288-292, https://doi.org/10.1016/j.meegid.2018.08.007.
6. Malhotra, B.; Singh, R.; Sharma, P.; Meena, D.; Gupta, J.; Atreya, A.; Meena, B.R. Epidemiological & clinical profile of influenza A (H1N1) 2009 virus infections during 2015 epidemic in Rajasthan. *Indian J Med Res.* **2016,** *144*, 918-923.
7. Kukhanova, M.K.; Korovina, A.N.; Kochetkov, S.N. Human herpes simplex virus: life cycle and development of inhibitors. *Biochemistry (Moscow)*, **2014,** *79*, 1635-1652, https://doi.org/10.1134/S0006297914130124.
8. Ludwig, S.; Zell, R.; Schwemmle, M.; Herold, S. Influenza, a One Health paradigm—novel therapeutic strategies to fight a zoonotic pathogen with pandemic potential. *Int. J. Med. Microbiol.* **2014,** *304*, 894-901, https://doi.org/10.1016/j.ijmm.2014.08.016.
9. Zhao, J.; Aisa, H.A. Synthesis and anti-influenza activity of aminoalkyl rupestonates. *Bioorganic Med. Chem. Lett.* **2012,** *22*, 2321-2325, https://doi.org/10.1016/j.bmcl.2012.01.056.
10. Oves, M.; Aslam, M.; Rauf, M.A.; Qayyum, S.; Qari, H.A.; Khan, M.S.; Alam, M.Z.; Tabrez, S.; Pugazhendhi, A.; Ismail, I.M. Antimicrobial and anticancer activities of silver nanoparticles synthesized from the root hair extract of Phoenix dactylifera. *Mater. Sci. Eng. C.* **2018,** *89*, 429-443, https://doi.org/10.1016/j.msec.2018.03.035.
11. Ibrahim, H.M. Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms. *J. Radiat. Res. Appl. Sci.* **2015,** *8*, 265-275, https://doi.org/10.1016/j.jrras.2015.01.007.
12. 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*, 137-142, https://doi.org/10.1016/j.jviromet.2011.09.003.
13. Lu, L.; Sun, R.W.; Chen, R.; Hui, C.K.; Ho, C.M.; Luk, J.M.; Lau, G.K.; Che, C.M. Silver nanoparticles inhibit hepatitis B virus replication. *Antiviral therapy* **2008,** *13*.
14. Lara, H.H.; Ayala-Nuñez, N.V.; Ixtepan-Turrent, L.; Rodriguez-Padilla, C. Mode of antiviral action of silver nanoparticles against HIV-1. *J. Nanobiotechnology* **2010,** *8*, 1-10, https://doi.org/10.1186/1477-3155-8-1.
15. Sharma, V.; Kaushik, S.; Pandit, P.; Dhull, D.; Yadav, J.P.; Kaushik, S. Green synthesis of silver nanoparticles from medicinal plants and evaluation of their antiviral potential against chikungunya virus. *App Microbio Biotech.* **2019,** *103*, 881–891, https://doi.org/10.1007/s00253-018-9488-1.
16. Hu, R.L.; Li, S.R.; Kong, F.J.; Hou, R.J.; Guan, X.L.; Guo, F. Inhibition effect of silver nanoparticles on herpes simplex virus 2. *Genet Mol Res.* **2014,** *13*, 7022-7028, https://dx.doi.org/10.4238/2014.March.19.2.
17. Xiang, D.; Zheng, Y.; Duan, W.; Li, X.; Yin, J.; Shigdar, S.; O'Connor, M.L.; Marappan, M.; Zhao, X.; Miao, Y.; Xiang, B.; Zheng, C. Inhibition of A/Human/Hubei/3/2005 (H3N2) influenza virus infection by silver nanoparticles *in vitro* and *in vivo*. *Int J Nanomedicine* **2013**, *8*, 4103–4113, https://doi.org/10.2147/IJN.S53622.
18. El-Mohamady, R.S.; Ghattas, T.A.; Zawrah, M.F.; Abd El-Hafeiz, Y.G.M. Inhibitory effect of silver nanoparticles on bovine herpesvirus-1. *Int J Vet Sci Med.* **2018**, *6*, 296-300, https://doi.org/10.1016/j.ijvsm.2018.09.002.
19. Deepak, A.K. Synthesis of aminoacid conjugate of tris(hydroxymethyl)phosphine and nanoparticle composition thereof. **2022;** P.No.367516.
20. Ashokkumar, T.; Prabhu, D.; Geetha, R.; Govindaraju, K.; Manikandan, R.; Arulvasu, C.; Singaravelu, G. Apoptosis in liver cancer (HepG2) cells induced by functionalized gold nanoparticles. *Colloids and Surfaces B: Biointerfaces* **2014,** *123*, 549-556, https://doi.org/10.1016/j.colsurfb.2014.09.051.
21. Gaikwad, S.; Ingle, A.; Gade, A.; Rai, M.; Falanga, A.; Incoronato, N.; Russo, L.; Galdiero, S.; Galdiero, M. Antiviral activity of mycosynthesized silver nanoparticles against herpes simplex virus and human parainfluenza virus type 3. *Int. J. Nanomed* **2013,** *8*, https://doi.org/10.2147/IJN.S50070.
22. Orlowski, P.; Tomaszewska, E.; Gniadek, M.; Baska, P.; Nowakowska, J.; Sokolowska, J.; Nowak, Z.; Donten, M.; Celichowski, G.; Grobelny, J.; Krzyzowska, M. Tannic acid modified silver nanoparticles show antiviral activity in herpes simplex virus type 2 infection. *PloS one* **2014,** *9*, https://doi.org/10.1371/journal.pone.0104113.

23. Dhanasezhian, A.; Srivani, S.; Govindaraju, K.; Parija, P.; Sasikala, S.; Kumar, M.R. Anti-Herpes Simplex Virus (HSV-1 and HSV-2) activity of biogenic gold and silver nanoparticles using seaweed *Sargassum wightii*. *Indian J Mar Sci.* **2019,** *45*, 1252–1257.
24. Jeremiah, S.S.; Miyakawa, K.; Morita, T.; Yamaoka, Y.; Ryo, A. Potent antiviral effect of silver nanoparticles on SARS-CoV-2. *Biochem Biophys Res Commun*. **2020,** *533*, 195-200, https://doi.org/10.1016/j.bbrc.2020.09.018.
25. Morris, D.; Ansar, M.; Speshock, J.; Ivanciuc, T.; Qu, Y.; Casola, A.; Garofalo, R.P. Antiviral and immunomodulatory activity of silver nanoparticles in experimental RSV infection. *Viruses* **2019,** *11*, https://doi.org/10.3390/v11080732.