International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 15, Issue 6, 2023

Original Article

SILVER NANOPARTICLES INHIBIT INFECTIOUS BRONCHITIS VIRUS REPLICATION

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Received: 25 Jul 2023, Revised and Accepted: 13 Sep 2023

ABSTRACT

Objective: Avian infectious bronchitis virus (IBV) threatens the poultry industry and causes global economic losses. The IBV is highly variable. Thus, no effective drugs are available. Objective of the present study was to evaluate silver nanoparticles against it as an antiviral agent.

Methods: Silver nanoparticles (AgNPs) have been evaluated as antivirals against IBV. *P. betle* leaf extract biosynthesizes AgNPs from silver nitrate. UV/vis absorption, X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and transmission electron microscopy (TEM) have been used to characterize AgNPs. TEM indicated particle sizes of 5–30 nm, and XRD demonstrated their characteristic AgNPs structure. The antiviral activity of AgNPs was measured by the log embryo infective dose 50 ($logEID_{50}$)/ml and the number of IBV genome copies.

Results: XRD analysis showed a structure for AgNPs, and transmission electron microscopy showed a size of 5–30 nm for AgNPs. AgNPs at a noncytotoxic concentration inhibit the interaction between the virus and the cell, preventing the virus from entering the cell and reducing the number of IBV genome copies (per µl) in ovo by preventing the formation of the IBV RNA genome, resulting in a significant reduction in the IBV titer.

Conclusion: AgNPs possess antiviral properties that inhibit IBV replication in ovo. The findings indicate that AgNPs are a promising drug candidate for treating or preventing IBV infection.

Keywords: Silver nanoparticles, Antiviral activity, Infectious bronchitis virus, IBV genomes, Coronaviruses

DOI: https://dx.doi.org/10.22159/ijap.2023v15i6.48963. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

The poultry industry plays a significant role in food production and poverty alleviation. However, the spread of infectious diseases among birds, such as the avian infectious bronchitis virus (IBV), poses a threat to poultry health and results in economic losses [1]. Additionally, these pathogens can cause zoonotic diseases, further impacting human health [2]. IBV, the coronavirus of the chicken, is a family of enveloped, non-segmented, single-stranded, positive-sense ribonucleic acid (+ssRNA) viruses [3].

Traditional antiviral treatments for IBV often lead to resistance, side effects, and the recurrence of viruses [4]. Vaccination is commonly used, but the constant emergence and re-emergence of viral strains can compromise its efficacy [5-9]. To address these challenges, researchers are exploring alternative strategies, including the use of medicinal plant derivatives and nanotechnology, to develop novel antiviral drugs.

P. betle leaves have been used for centuries in traditional medicine and cultural practices. These leaves are rich in various bioactive compounds, including polyphenols such as acetyl eugenol, trans-isoeugenol, chavicol, chavibetol, chavibetol acetate, and allyl pyrocatechol diacetate [10]. These polyphenols have attracted significant attention due to their potential applications in various industries, including the preparation of silver nanoparticles (AgNPs) [10]. Polyphenols present in P. betle leaves can act as reducing and capping agents on the surface of AgNPs. This means that they can prevent the aggregation of nanoparticles and provide numerous benefits by reducing the size of AgNPs. Polyphenols enhance their stability and improve their overall performance [10].

AgNPs can attach to the viral genome and disrupt the viral envelope, rendering the virus inactive and unable to infect host cells or replicate. AgNPs increase viral particle contact due to their small size and large surface area [10]. Therefore, In this study, aqueous extracts of *P. betle* were used to produce AgNPs and were characterized by UV/vis absorption, X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and transmission electron microscopy (TEM). Also, we evaluated the *in ovo* antiviral effect of AgNPs on IBV infectivity and genome replication.

MATERIALS AND METHODS

Plant material

P. betle leaves were obtained from Malaysia's main vegetable markets. The Department of Botany, Faculty of Pharmacy, Amman, Jordan, identified and authenticated the leaves. A voucher specimen (PJ 05) was deposited.

Synthesis of AgNPs

P. betle aqueous extract was described previously (10), by mixing 10 g of a dry leaf sample with 100 ml of highly purified water in a flask. For 72 h at 8 °C, the flask was plugged with cotton wool. Filtering the mixture with 25-mm Whatman filter paper removed plant residues [9]. The pellets were vacuum-dried and stored at room temperature for analysis. Pellets are stored at-20 °C until use. AgNPs were made biologically [5, 10]. In brief, 10 ml of *P. betle* aqueous leaf extract (10 g/100 ml) was mixed with a 5 mmol silver nitrate solution and left at room temperature (25 °C) for four hours before centrifugation at 10,000 rpm for ten minutes. After discarding the supernatant, the residue was washed with distilled water (7000 rpm, 2 min) and vacuum-dried. Before analysis, dried pellets needed to be at room temperature [10]. After removing the supernatant, the residue was washed with distilled water at 7000 rpm for 2 min and vacuum-dried. Before analysis, dried pellets had to be stored at room temperature [10].

Characterization of AgNPs

The AgNP characterization was described previously [10]. Briefly, Ultraviolet/visible spectrophotometry examined synthesized AgNPs. AgNPs absorb surface plasmon resonance at 420 nm. We analyzed the dried AgNPs composite with a 40 kV X'Pert Pro XRD (PANalatical Empyrean-2012). The GBC-Difftech MMA model analyzed dried AgNPs' structural composition. Radiation was 34.2 mA and 35 kV Ni-filtered Cu Ka (k = 1.54 A °). TEM measured AgNPs sizes. Drop coated AgNPs onto carbon-coated copper TEM grids prepared for samples. Blotting the films on the TEM grid after two minutes removed excess solution [10]. The grid was dried prior to the measurements.

Anti-viral activity assay *in vivo*

Antiviral activity was described previously [11, 12]. Briefly, we propagated IBV-H120 in embryonated Specific-Pathogen-Free (SPF) chicken eggs. After obtaining the master seed of the IBV-H120 vaccine strain (Jordan Bio-Industries Center (JOVAC), Amman, Jordan) with 10^8 embryo infective dose 50 (EID_{50})/ml, we diluted it in 1 ml of sterile phosphate buffer saline (PBS; pH 7.2) and serially diluted it until we reached 10^5 EID_{50}/ml. At day 10, SPF chicken eggs were inoculated with 0.1 ml of 10^5 EID_{50}/ml in the presence of different concentrations of AgNPs and incubated at 37 °C for 1 h [11, 12].

The SPF chicken eggs were incubated at 37 °C with 60% humidity. SPF chicken eggs were incubated daily with candlelight for 7 d. First-day deaths are nonspecific. The eggs' tops were removed, and allantoic fluids were collected 7 d post-infection. Allantoic fluids were sucked without albumin or yolk.

We immediately refrigerated all fluids at 4 °C [11, 12]. Control (0.1 ml 10^5EID_{50}/ml of IBV-H120 virus without treatments) and IBV-infected samples were titrated using the SPF chicken eggs to calculate EID_{50}/ml values [11, 12].

Viral titration for IBV-H120

To measure viral EID_{50}/ml titers, we injected 100 µl of 10-fold virus dilutions (5 eggs/dilution) into the allantoic cavities of 10 d old SPF chicken eggs [10].

Viral RNA extraction and qRT-PCR

The NZY Viral RNA Isolation Kit (NZY Tech, Lisbon, Portugal) was used to extract RNA from allotonic fluid. Each RNA sample's reaction mixture was prepared per the manufacturer's instructions.

IBV One-Step RT-qPCR Kit, RUO (NZY Tech, Portugal), amplified ORF1a. Quantitative analysis used a standard curve.

Statistical analysis

GraphPad Prism and SPSS performed all analyses. One-way ANOVA with post-hoc Tukey's multiple comparisons determined group differences. $P < 0.05$ was significant.

RESULTS

Characterization of AgNPs

Sample absorbance indicates increased reaction mixture AgNPs production. AgNPs also changed color to dark brown (fig. 1). FTIR data identified biomolecule functional groups involved in bioreduction and capping for efficient AgNPs stabilization (fig. 2). TEM measured the AgNPs at 5-30 nm (fig. 3A). XRD patterns show that AgNPs have mixed cubic and hexagonal structures and an average particle size of 20 nm. The XRD pattern has three peaks for all values between 10 and 80. Fig. 3B shows multiple Bragg reflections with values of 38.52, 46.529, and 64.53 for AgNPs (111), (200), and (220) facets.

Cytotoxicity of AgNPs

Injecting 10-day-old SPF chicken eggs with 360 µg/ml AgNPs did not cause toxicity (fig. 4). Due to solvent toxicity, high-concentration eggs are difficult to inject. So, injecting eggs with more than 1500 µg/egg is impractical. Therefore, AgNPs' median lethal dose (LD_{50}) was not calculated.

Anti-viral activity of AgNPs against IBV-H120 *in ovo*

The $LogEID_{50}$/ml was determined by pooling the allantoic fluids from all of the eggs that had been harvested 7 d after infection. AgNPs inhibit the replication of IBV-H 120 in a dose-dependent manner, as shown by a decrease in $LogEID_{50}$/ml. *In ovo*, Significant inhibitory activity against infectious IBV-H120 was observed for AgNPs at 10, 20, 40, 80, 160, and 320 µg/egg ($p < 0.001$) (fig. 5).

AgNPs inhibit IBV genome synthesis

The copy number (per µl)/quantitation Cycle (Cq) value in allantoic fluids showed a significant decrease in IBV genome synthesis *in ovo* (table 1).

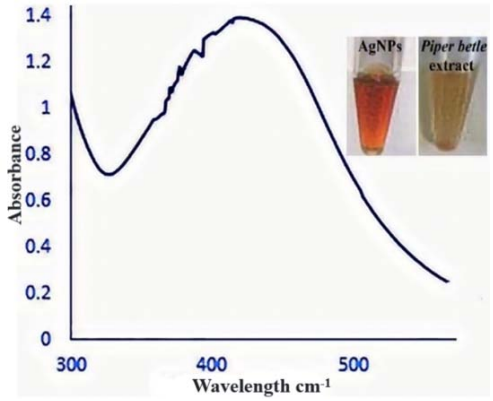

Fig. 1: AgNPs surface plasmon resonance absorption at 420 nm

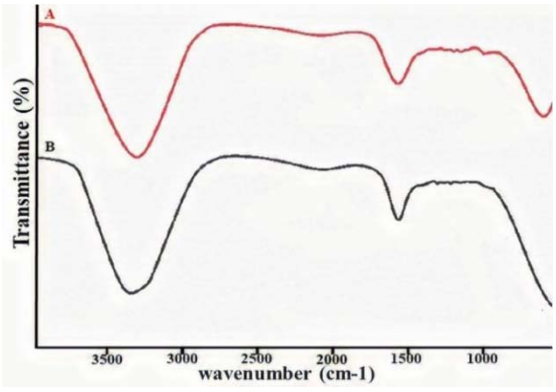

Fig. 2: FTIR spectra of (A) leaf extract of *P. betle* extraxt (B) AgNPs synthesized by using *P. betle* extract

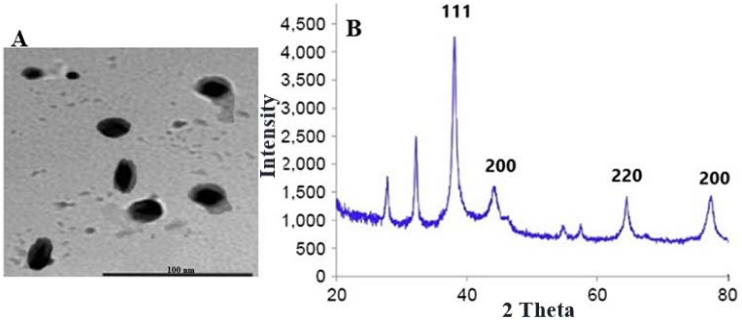

Fig. 3: (A) TEM analysis of AgNPs (B) XRD pattern of synthesized AgNPs

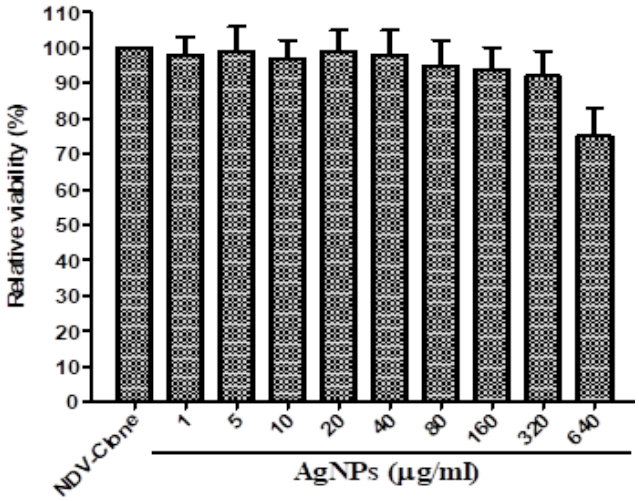

Fig. 4: Cytotoxicity of AgNPs. The data are presented as the mean±standard deviation (SD) of triplet samples

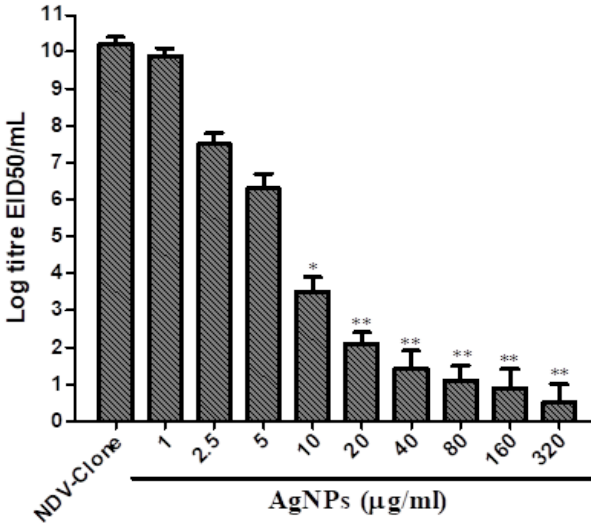

Fig. 5: AgNPs anti-IBV efficacy in ovo: (A) In ovo AgNPs anti-IBV efficacy: 10 d old SPF chicken eggs were infected with IBV at 0.1 ml of 10^5 EID_{50}/ml in different AgNPs concentrations and observed daily for embryo mortality for 7 d. Pooling all egg allantoic fluid after harvest. Using Reed-Muench, calculate EID_{50}/ml. The data are presented as the mean±SD of triplet samples, *p<0.01, **p<0.001

Table 1: AgNPs affect IBV genome quantification in allantoic fluids. After infecting SPF embryonated chicken eggs (day 10) with IBV (0.1 ml of 10^5 EID_{50}/ml) with different levels of AgNPs, embryo mortality was recorded daily for 7 d. Extracting all eggs' allantoic fluids. qRT-PCR measured allantoic fluid IBV genome concentration. *p<0.01; **p<0.001

AgNPs (µg/ml)	Copy number (per µl)	*P* value
0 as control	$3x10^5$	
20	$5x10^2$	
40	$2x10^2$	**
80	$8x10^1$	**
160	$3x10^1$	**
320	$2x10^1$	**

DISCUSSION

AgNPs have gained attention in recent years due to their antimicrobial properties. These nanoparticles, typically less than 100 nanometers in size, possess a large surface area that enhances their interaction with viruses [13]. The unique properties of AgNPs allow them to interact with a wide range of viruses, including those that cause bronchitis. They have been shown to be effective against respiratory syncytial virus, influenza virus, and other common bronchitis viruses [14]. This study agrees with several studies that demonstrate AgNPs can inhibit early infection through the direct interaction between AgNPs and the viral envelope [15-17]. AgNPs can disrupt the integrity of the viral envelope, preventing the virus from entering host cells and replicating [15]. Additionally, AgNPs have been found to have a low risk of inducing viral resistance, making them a potentially long-term solution for combating viral infections [16, 17].

We demonstrated that AgNPs exhibited a robust inhibition of RNA synthesis; the most likely explanation is through direct Ag binding to

RNA polymerase [18]. Similarly, the antiviral activity of AgNPs has been investigated against various viruses, including avian influenza (AI) H9N2 [15] and Newcastle disease virus (NDV). In the case of AI H9N2, the nanoparticles have shown potential for inhibiting viral entry and reducing viral replication [13]. These different mechanisms of action make AgNPs a potent antiviral agent with the ability to disrupt multiple stages of the viral life cycle.

The unique physicochemical properties of AgNPs enable them to interact with viral particles in a way that makes it difficult for the microbe to adapt and develop resistance [18-20]. This makes green AgNPs a valuable tool in combating viral infections that are resistant to traditional antiviral drugs.

CONCLUSION

The use of AgNPs synthesized using *P. betle* aqueous extract represents a breakthrough in the field of antiviral therapy. These AgNPs exhibit potent antiviral activity against IBV. By inhibiting viral entry and replication, AgNPs offer a promising alternative to traditional antiviral drugs. Furthermore, their ability to overcome microbial resistance makes them valuable in combating viral infections that are resistant to conventional treatments. Further research and development in this field will pave the way for the application of AgNPs in the prevention and treatment of viral infections.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

There is no conflict of interest.

REFERENCES

1. Nieto Torres JL, DeDiego ML, Verdia Baguena C, Jimenez Guardeno JM, Regla Nava JA, Fernandez Delgado R. Severe acute respiratory syndrome coronavirus envelope protein ion channel activity promotes virus fitness and pathogenesis. PLOS Pathog. 2014;10(5):e1004077. doi: 10.1371/journal.ppat.1004077, PMID 24788150.
2. Schneider K, Klaas R, Kaspers B, Staeheli P. Chicken interleukin-6. cDNA structure and biological properties. Eur J Biochem. 2001;268(15):4200-6. doi: 10.1046/j.1432-1327.2001.02334.x, PMID 11488913.
3. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG. A new coronavirus associated with human respiratory disease in China. Nature. 2020;579(7798):265-9. doi: 10.1038/s41586-020-2008-3, PMID 32015508.
4. Legnardi M, Tucciarone CM, Franzo G, Cecchinato M. Infectious bronchitis virus evolution, diagnosis and control. Vet Sci. 2020;7(2):79. doi: 10.3390/vetsci7020079, PMID 32580381.
5. Guzman M, Hidalgo H. Live attenuated infectious bronchitis virus vaccines in poultry: modifying local viral populations dynamics. Animals (Basel). 2020;10(11):2058. doi: 10.3390/ani10112058, PMID 33171704.
6. Saadh MJ. SARS-COV-2 3CL-protease inhibitors as antiviral agent against COVID-19. Int J App Pharm. 2022;14(6):18-20, doi: 10.22159/ijap.2022v14i6.46015.
7. Saadh MJ, Almaaytah AM, Alaraj M, Dababneh MF, Sa'adeh I, Aldalaen SM, Kharshid AM, Alboghdadly A, Hailat M, Khaleel A, Al-Hamaideh KD. Punicalagin and zinc (II) ions inhibit the activity of SARS-CoV-2 3CL-protease *in vitro*. Eur Rev Med Pharmacol Sci. 2021;25(10):3908-13. doi: 10.26355/eurrev_202105_25958.
8. Sukumaran S, Sathianarayanan S. A review on COVID-19 pandemic a global threat-current status and challenges and preventive strategies. Int J App Pharm. 2021;13(5):10-4, doi: 10.22159/ijap.2021v13i5.42070.
9. Saadh MJ. Omicron sars-Cov-2 variant, b.1.1.529: severity, transmission, mutation, and efficacy of current vaccines. Int J App Pharm 2022;14:12-5. doi: 10.22159/ijap.2022v14i3.44376.
10. Saadh MJ. Green synthesis of silver nanoparticles using *Piper betle* exhibit high antimicrobial activity against *Salmonella enteritidis* and *E. coli*. Iraqi J Vet Sci. 2023;37(3):759-63, doi: 10.33899/IJVS.2023.136475.2585.
11. Saadh MJ, Jaber SA, Alaraj M, Alafnan A. Apigenin inhibits infectious bronchitis virus replication in ovo. Eur Rev Med Pharmacol Sci. 2022;26(15):5367-71. doi: 10.26355/eurrev_202208_29403, PMID 35993630.
12. Saadh MJ, Aldalaen SM. Inhibitory effects of epigallocatechin gallate (EGCG) combined with zinc sulfate and silver nanoparticles on avian influenza a virus subtype H5N1. Eur Rev Med Pharmacol Sci. 2021;25(6):2630-6. doi: 10.26355/eurrev_202103_25427, PMID 33829450.
13. Karna SR, RK, ND. Anticancer activity of silver nanoparticle of prodigiosin on lung cancer. Int J App Pharm. 2023;15(1):264-8, doi: 10.22159/ijap.2023v15i1.43628.
14. Nefedova E, Koptev V, Bobikova AS, Cherepushkina V, Mironova T, Afonyushkin V. The infectious bronchitis coronavirus pneumonia model presenting a novel insight for the SARS-CoV-2 dissemination route. Vet Sci. 2021;8(10):239. doi: 10.3390/vetsci8100239, PMID 34679068.
15. Saadh MJ, Aggag MM, Alboghdadly A, Kharshid AM, Aldalaen SM, Abdelrazek MA. Silver nanoparticles with epigallocatechingallate and zinc sulphate significantly inhibits avian influenza virus H9N2. Microb Pathog. 2021;158:(105071). doi: 10.1016/j.micpath.2021.105071, PMID 34182075.
16. Saadh M. Potent antiviral effect of green synthesis silver nanoparticles on Newcastle disease virus. Arab J Chem. 2022;15(7):103899. doi: 10.1016/j.arabjc.2022.103899.
17. Saadh MJ. Silver nanoparticles inhibit goatpox virus replication. Arch Virol. 2023;168(1):32. doi: 10.1007/s00705-022-05667-5, PMID 36604362.
18. Galdiero S, Falanga A, Vitiello M, Cantisani M, Marra V, Galdiero M. Silver nanoparticles as potential antiviral agents. Molecules. 2011;16(10):8894-918. doi: 10.3390/molecules16108894, PMID 22024958.
19. Muhaimin M, Chaerunisaa AY, Rostinawati T, Amalia E, Hazrina A, Nurhasanah S. SA review on nanoparticles of *Moringa oleifera* extract: preparation, characterization, and activity. Int J App Pharm. 2023;15(4):43-51. doi: 10.22159/ijap.2023v15i4.47709.
20. Gupta MO. Biosynthesized silver nanoparticles using *Catharanthus roseus* and their antibacterial efficacy in synergy with antibiotics; a future advancement in nanomedicine. Asian J Pharm Clin Res. 2021;14(2):116-24, doi: 10.22159/ajpcr.2021.v14i2.39856.