RESEARCH PAPER | Microbiology | Volume : 5 | Issue : 6 | June 2015 | ISSN - 2249-555X

Cytotoxic and Anti Influenza Activity 0f Silver Nanoparticles from *Withania Somnifera*

KEYWORDS: Silver nanoparticles, Anti- influenza activity, Cytotoxicity, Green Chemistry, Withania somnifera.

V. N. Magare	Charuu P. Kulkarnii	Chandra B. Maurya
Principal, Dept. of Zoology, Kirti M. Doongursee College of Arts, Science and Commerce, Dadar (W), Mumbai	Dept. of Chemistry, Kirti M. Doongursee College of Arts, Science and Commerce, Dadar (W), Mumbai	Dept. of Chemistry, G.N. Khalsa College of Arts, Science & Commerce, Matunga (E), Mumbai

R. C. Patil	Sandesh S. Mandavkar
Dept. of Microbiology, Bhavan's College, Andheri (W), Mumbai	Dept. of Microbiology, Bhavan's College, Andheri (W), Mumbai

ABSTRACT *Silver nanoparticles have been used in a wide range of products, most importantly as antimicrobial agents. Silver nanoparticles are also used in hygienic products including water purification systems, linings of washing machine, dishwashers, refrigerators, and toilet seats. The present study reports the synthesis of silver nanoparticles from fresh leaves of Withania somnifera using a chemical method. After successful synthesis and characterization, these Ag-NPs were evaluated for their anti-influenza potential. Silver nanoparticles synthesized using Withania somnifera in the current study showed promising anti-influenza activity.*

INTRODUCTION

Silver has been in use since time immemorial in numerous forms for the treatment of burns, wounds and several bacterial infections. However, due to the emergence of several antibiotics the use of these silver compounds has been declined remarkably. Nanotechnology is gaining tremendous importance in the present century due to its capability of modulating metals into their nano-size, which drastically changes the chemical, physical and optical properties of metals. Silver nanoparticles have become a remarkable antimicrobial agent. With the emergence of new antibiotic drug resistant bacterial strains, the use of silver nanoparticles is gaining considerable attention. Hence, silver nanoparticles have emerged up with diverse medical applications (Rai et al., 2009).

Green chemistry or green synthesis of silver nanoparticles involves use of plants for the use of silver nanoparticles. This method is fast, efficient and eco – friendly method for synthesis of silver nanoparticles. Many medicinal plants have been used for the synthesis of silver nanoparticles (Patil et al., 2013; Ahmed et al., 2015).

Withania somnifera (Family- Solanacae), also known as Ashwagandha, Indian ginseng, or winter cherry, has been an important herb in the Ayurvedic and indigenous medical systems for over 3000 years (Sandhu et al., 2010). The extract of *Withania somnifera* has been reported to have analgesic, mildly sedative, anti-inflammatory and anabolic activities and is useful in stress, strain, fatigue, pain, skin diseases, diabetes, gastrointestinal disease, rheumatoid arthritis, epilepsy, chronic fatigue syndrome and even during pregnancy without any side effects (Weiner and Weiner, 1994; Prakash et al., 2002; Singh et al., 2002). It is also used as a general tonic, to increase energy and improve health and longevity (Mishra et al., 2000).

Though there are number of reports available highlighting the medicinal properties of *Withania somnifera*, its efficacy against Influenza virus remains unreported. In view of this, the present study was undertaken to generate Ag-NPs and evaluate their cytotoxic and anti-influenza potential.

MATERIALS AND METHODS

1. Preparation of Test Samples

Finely ground dry powder of roots of *Withania somnifera* was subjected to Soxhlet extraction method. Hot aqueous extract was prepared using water as the solvent.

Fresh leaves of *W. somnifera* were collected from a pond in Tamilnadu and then extract was prepared following a method described by Sinha et al., (2009) and Patil et al., (2013), first the filtrate was prepared and then this filtrate was treated with 1mM silver nitrate and silver nanoparticles were synthesized.

Silver nanoparticles were also prepared by using the wet chemical reduction method using 1mM silver nitrate solution and 1% sodium citrate solution as metal salt precursor and stabilizer respectively (Guzman et al., 2008).

2. Cell culture

Madin Darby Canine Kideny (MDCK) cell line was procured from Haffkine Institute, Mumbai and used for the isolation of influenza virus. Cells were maintained in MEM medium supplemented with 10% FBS, 1% antibiotic-antimycotic solution at 37°C in a humidified 5% CO_2 incubator. The cell line was routinely subcultured upon reaching 70-80% confluency (i.e. 2-3 times in a week). Assessment of cell viability and viable cell counting was carried out using trypan blue dye exclusion method.

Volume : 5 | Issue : 6 | June 2015 | ISSN - 2249-555X

3. Cytotoxicity assay

MDCK cells suspended in complete medium were seeded in a volume of 100 µl per well in a 96 well microtitre plate and the plate was incubated in a humidified 5% CO_2 incubator at 37°C for 24 hr to allow complete monolayer formation. Once the monolayer was formed, the complete medium was removed from the well and 100 µl of desired concentrations of test solutions were added. A vehicle control (MEM with 1% DMSO), a positive control (cells without test solution) and negative control (cells with 100% DMSO) were also maintained along with the test samples. The plate was then incubated at 37°C in 5% CO_2 for 16 – 18 hr. After incubation the samples were removed using multichannel pipette and 10% of 5 mg/ml MTT reagent (100 µl) was added in each well. The plates were incubated at 37°C for 4 hr. After incubation, MTT was removed and 100 µl of DMSO was added in each well. The plates were further incubated on shaker for 1 min and then read on ELISA plate reader at 550 nm.

4. Anti - influenza assay

The aim of this assay was to determine the effect of silver nanoparticles on virus growth and its replication. The effect of the silver nanoparticles was assessed by carrying out simultaneous treatment assay.

Before starting with the assay, virus growth medium was prepared by adding 0.2 ml of Tosyl Phenylalanyl Chloromethyl Ketone (TPCK), Trypsin (final concentration 2µg/ml), 0.2 ml Nystatin (final concentration 50 units/ml) into total 50 ml sterile MEM medium. The standard virus aliquot was taken from -80°C deep freezer, thawed properly and used for the anti influenza activity.

In simultaneous exposure assay, 50 µl of the virus and 50 µl of different dilutions of the silver nanoparticles prepared in virus growth medium were added simultaneously to MDCK cells and the plate was kept in 37°C in 5% CO_2 incubator.

The dilutions of the test samples used for the assay were: undiluted (neat), 1:2, 1:20, 1:200, 1:2000 and 1:10,000. After addition of test samples in simultaneous assay, the plate was incubated at 37°C, 5% CO_2 for 72 hr.

FINDINGS

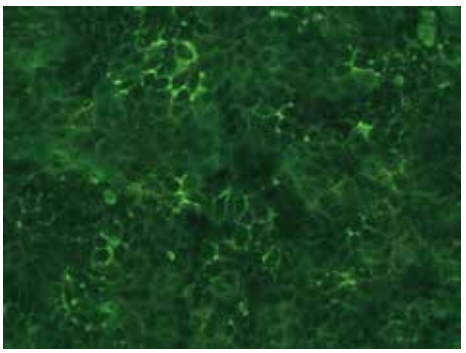
1. Cytotoxicity Assay

In cytotoxicity assay, the chemically synthesized silver nanoparticles and plant extract did not show any cytotoxic effect and hence their CC50 was not calculated. On the other hand, silver nanoparticles synthesized by *Withania somnifera* were found to be cytotoxic and their CC50 was found to be 85µg/ml and it was selected for antiviral activity.

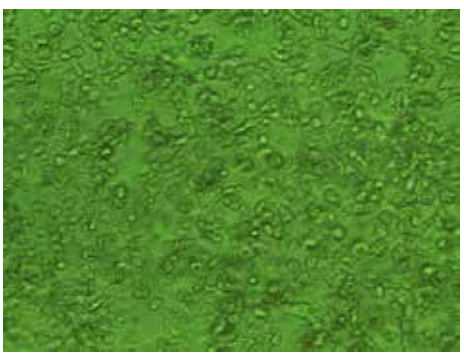
2. Anti - Influenza assay

On incubation at 37°C for 96 h in 5% CO_2 incubator, the monolayer was observed for cytopathic effect. Cell control showed expected cytopathic effect. No cytopathic effect was seen indicating anti – influenza activity of silver nanoparticles synthesized by *Withania somnifera* (Figure 1) and surprisingly cytopathic effect was seen in case of chemically synthesized silver nanoparticles indicating no effect against influenza virus.

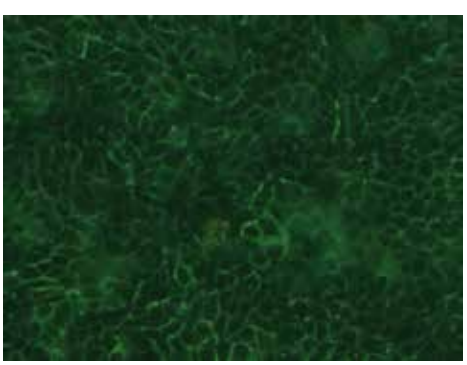
Figure 1 Results of Anti – Influenza activity



MDCK cell line inoculated with virus and silver nanoparticles synthesized by wet chemical reduction method before incubation



MDCK cell line inoculated with virus and silver nanoparticles synthesized by wet chemical reduction method after incubation showed cytopathic effect.



MDCK cell line inoculated with virus and silver nanoparticles synthesized by Withania somnifera before incubation

MDCK cell line inoculated with virus and silver nanoparticles synthesized by Withania somnifera after incubation did not show any cytotoxic effect

Volume : 5 | Issue : 6 | June 2015 | ISSN - 2249-555X

SUMMARY

The above results clearly highlight that the silver nanoparticles synthesized from the fresh leaves *Withania somnifera* have a promising anti - influenza activity and therefore likely to have many more beneficial prophylactic and therapeutic effects.

ACKNOWLEDMENTS

Authors would like to express their sincere thanks to the authorities of Haffkine Research Institute, Parel, Mumbai for providing facilities for this study.

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