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Antiviral properties of silver nanoparticles synthesized by *Aspergillus sps*

G. Narasimha*[1], Habeeb Khadri[2] and Mohammed Alzohairy[2]

[1]Department of Virology, Sri Venkateswara University, Tirupati, A.P, India
[2]Department of Medical Sciences, College of Applied Medical Sciences, Qassim University, Qassim, Kingdom of Saudi Arabia

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ABSTRACT

An invitro study was conducted to investigate the antiviral properties of silver nanoparticles synthesized from fungal strain Aspergillus sps isolated from soil. The isolated and characterized silver nanoparticles exhibited as an excellent antiviral property on Bacteriophage viral strain. The viral inactivation process was increased with increasing the concentration of silver nanoparticles.

Key words: *Aspergillus sps, Silver nanoparticls, E.coli, Bacteriophage, Antiviral activity.*

INTRODUCTION

In present days, resistance to commercially available antimicrobial agents by pathogenic microorganisms has been increasing at an alarming rate and has become a serious problem. There is need to search for novel antimicrobial and antiviral agents from natural and inorganic substances. The inorganic agent silver has been employed as most antimicrobial agent, since ancient times to fight infections pathogens [1]The significant feature of silver is its broad spectram antimicrobial property which is due to microbial colonization associated with biomaterial related infections[2] . There are many invitro studies on antibacterial and antifungal properties of silver nanoparticles. But the reports on antiviral activity of silver nanoparticles was scanty. Hence there is need for much research on antiviral compounds including inorganic, organic and metallic nanoparticles from chemical and biological systems for control of viral diseases in plants, animals, and human beings. In this study, an attempt was made on antiviral properties of silver nanoparticles synthesized from soil fungi *Aspergillus sps.*

The viruses are obligative intracellular pathogenic agents in both eukaryotes and prokaryotes, the only real link between viral bacteriophages and actual human pathogens, is their ability to alter the genome of non-virulent bacteria strains, producing more virulent strains. Previous reports made on antiviral activity of chemical agents iodine and chlorine dioxide against viral strains bacteriophag and poliovirus and concluded that oxidative damage of sulfhydryl groups in the protein coat was an important aspect in the killing mechanism of nanoparticles [3]. Elechiguerra, (2005) [4] reported that, silver nanoparticles with very small sizes are susceptible to Human Immuno virus, binding of silver nanoparticles of size less than 5nm to gp120 protein of HIV virus prevented the virus from attaching itself to the host tissue cells. The indications for use of a novel class of anti-HCV agent and exact antiviral mechanism of metallic nanoparticles may lead to the development of agents with potent activities against viruses [5].

MATERIALS AND METHODS

Collection of silver nanoparticles

The silver nanoparticles used in this study was synthesized from fungal strain *Aspergillus* their size and shapes were reported [6]

Isolation of viral host (*E.coli*)

The viral host, bacterial strain (*E.coli*) was isolated from sewage water and the the culture was isolated on EMB agar medium by streak plate method under sterile conditions. After incubation the agar plates were incubated in incubator at 37 ^{0}C . After incubation, the bacterial colonies with metallic shine (unique nature of *E.coli*) were observed then transferred to nutrient broth and kept for shaking for preparing *E.coli* suspension. The viral host *E.coli*, bacterial strains were cultured in TGYE medium with following chemical ingredients g/L (Tryptone; 10, Glucose; 10, Yeast extract; 1, NaCl; 8. Typical viral phage preparations contain approximately 1X10^7-10^{11} cfu/ml.

Enrichment of Bacteriophages

The bacteriophages were enriched by standard methods [7]. For this a known volume of sewage water was transferred to conical flask; 5ml of 10X nutrient broth was transferred. This preparation was kept for mechanical shaking for 5-6 hrs at room temperature.

Inactivation of virus with nanoparticles

The isolated viral strains in the sewage samples were treated with the silver nanoparticles with various concentrations (30-240ppm) prepared and treated with virus particles in suspension and the mixture was vortexed and incubated.

Plaques formation on medium

The bacteriophage viral suspension (treated and without treated nanoparticles) and the *E.coli* suspension were mixed in soft agar medium and poured into replicative plates. After medium solidification is over the plates were incubated at 37^{0}C for 24 to 48 hrs in incubator. After incubation, the plates were observed for formation of plaques (Bacterial cell lysis) and the number of plaques was counted.

RESULTS AND DISCUSSION

The antiviral properties of silver nanoparticles isolated from soil fungi *Aspergillus sps* on bacteriphage was studied and the results were reported in table.1. With increasing the nanoparticles concentration from 30-210 ppm the veridical property also increased. It is an indication of decreasing the plaques number on the medium. Various nanoparticle concentrations used in this study, the nanoparticles concentrations from 30-180ul range reduced the plaque number, whereas at 210-240ppm totally inhibited the viral growth in host (bacteria)which is indication of complete inhibition of viral replication (viral growth) in host.(table.1). Similarly an vitro studies have contributed to the understanding of possible mechanisms by which nanoparticles or metal oxides such as Arsenic, Antimony leads to induction of apoptosis, inhibition of growth and angiogenesis, modulation of cellular signaling pathways, perturbation of cellular redox status, and promotion of differentiation[8].The two primary mechanisms control the oxidant disinfection efficiency by hydroxyl radicals: [9] oxidation and disruption of the cell wall and membrane with resulting disintegration of the cell [10].The diffusion of antiviral agent into the cell where it may inactivate the enzymes, damage intracellular components, interfere with protein synthesis and DNA replication[11]. The lower surface to volume ratio of the viruses may provide greater rates of hydroxyl radical reaction with intracellular biological molecules compared with the larger bacterial cells. The relatively slow diffusion of hydroxyl radicals into viruses, and particularly bacterial cells, may be the cause of its low disinfection rate, and may limit its use as a disinfectant [12] The antiviral activity in the present study correlates with antimicrobial activity of silver nanoparticles from *Aspergillus niger* jaydev and Narasimha (2010) [13] and white button mushrooms (*Agaricus bisporus*) Narasimha et al (2011) [14]. Elechiguerra et al (2005) [4] reported that the silver nanoparticles with very small size are susceptible to bacteria and fungi and HIV, binding of silver nanoparticles of size equal /less than 5nm to gp 120 protein of HIV virus prevented the virus from attaching itself to the host tissue cells. Further research needs to be in-depth of work on antiviral properties of silver nanoparticles and their molecularchr mechanism on viral inhibition.

Table.1.Ant viral properties silver nanoparticles at various concentrations

Plate No	Silvernanoparticle suspension (in ppm)	No. of Plaques*
1	Without silvernanoparticles (Control)	120
2	30	72
3	60	54
4	90	34
5	120	18
6	150	9
7	180	2
8	210	ND
9	240	ND

Values represented in the table are mean of duplicates
Pfu plaque forming units
ND: Not detected

CONCLUSION

Silver nanoparticles synthesized from soil fungi, *Aspergillus sps* effectively inhibited the growth of Bacteriophage virus in host bacteria. The viral inactivation process was increased with increasing the concentration of nanoparticle is an indication of antiviral properties of silver nanopartilces.

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