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Virucidal properties of silver nanoparticles synthesized from white button Mushrooms (*Agaricus bisporus*)

ABSTRACT

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An in vitro study was conducted to know the virucidal properties of silver nanoparticles synthesized from locally available white button mushrooms (*Agaricus bisporus*). The characterized colloidal silver nanoparticles exhibited as an excellent virucidal property on viral strain Bacteriophage. The viral inactivation process was increased with increasing the concentration of colloidal silver nanoparticles. The silver nanoparticle at 140-160ppm concentration inhibited the viral growth in host bacterial strain.

Keywords: *White Mushrooms; Silver nanoparticles; E.coli, Bacteriophage; Virucidal activity.*

INTRODUCTION

In recent years, resistance to commercially available antimicrobial agents by pathogenic microorganisms has been increasing at an alarming rate and has become a serious problem. Microorganisms, such as bacteria, fungi, and viruses cause severe infections in human beings. There is a need to search for new antimicrobial and antiviral agents from natural and inorganic substances. Among the inorganic agents, silver has been employed as the most important antimicrobial agent, since ancient times to fight infections pathogens [1] The significant feature of silver is its broad spectrum antimicrobial property which is due to microbial colonization associated with biomaterial related infections [2] . There are many studies on antibacterial antifungal properties of silver nanoparticles. But the reports on antiviral activity of silver nanoparticles were 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 virucidal nature of silver nanoparticles synthesized from locally available white mushrooms (*Agaricus bisporus*).

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The viruses are obligative intracellular pathogenic agents in both eukaryotes and prokaryotes (bacteria) the only real link between bacteriophages and actual human pathogens, is their ability to alter the genome of non-virulent bacteria strains; thus, producing more virulent strains. Previous reports made on antiviral activity of chemical agents iodine and chlorine dioxide against viral strains bacteriophage and poliovirus and concluded that oxidative damage of sulfhydryl groups in the protein coat was an important aspect in the killing mechanism [3]. Similarly, Elechiguerra, (2005) [4] reported that, silver nanoparticles with very small sizes are susceptible to HIV 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].

EXPERIMENTAL

Collection of silver nanoparticles

The silver nanoparticles used in this study was synthesized from white button mushrooms and their size and shapes were characterized, antibacterial and antifungal activities also tested and reported in our previous study[6].

Isolation of viral host (E.coli)

The viral host, bacterial strain (*E.coli*) was isolated by taking a loopful of sewage water on EMB agar medium by streak plate method under sterile conditions. Then the agar plates were incubated in incubator at 37°C for colony development. 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.

Enrichment of Bacteriophage virus

The viral strain bacteriophage was 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.

Isolation of viral host (E.coli)

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 1×10^7 - 10^{11} cfu/ml.

Viral inactivation with nanoparticles

The Bacteriophage viral strains in the sewage samples were treated with the colloidal silver nanoparticles synthesized from mushrooms with different concentrations (20-160ppm) prepared and treated with virus particles in suspension and the mixture was vortexed and incubated.

Observation of plaque formation

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°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 virudical properties of colloidal silver nanoparticles on virus bacteriophage were studied and the results were represented in Table 1. With increasing the nanoparticles concentration from 20-160 ppm the virucidal property increased with indication of formation of decreasing the plaque number on the medium. Different nanoparticle concentrations used in this study, the nanoparticles concentrations from 20-120ul reduced plaque formation, whereas at 140-160ppm totally inhibited the viral growth in host bacterial strain which is indication of complete inhibition of viruses (viral growth) in the host. The inhibition of plaques on the medium may be due to inactivation

of viral growth or viral replication in *E.coli* bacterial host (Table 1).

Table 1. Virucidal properties of colloidal silver nanoparticles at various concentrations

Plat No.	Silver nanoparticle suspension (in ppm)	No. of Plaques
1	Without silver nanoparticles (Control)	112
2	20	89
3	40	45
4	60	27
5	80	18
6	100	11
7	120	3
8	140	ND
9	160	ND

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

Similarly an *in 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] diffusion of antiviral agent into the cell or particle where it may inactivate 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 of silver nanoparticles with small size (3-10nm) in the present study correlates with Jaydev and

Narasimha(2010) [13] . Elechiguerra *et al.* (2005) [4]. The silver nanoparticles with very small size are susceptible bacteria and fungi and HIV virus, 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 work needs to be investigating the more studies on virucidal properties of silver nanoparticles and their molecular mechanism on viral inhibition.

CONCLUSIONS

Silver nanoparticles synthesized from white button mushrooms (*Agaricus bisporus*) effectively inhibited the growth of Bacteriophage viral strain in host bacterial strain. The viral inactivation process was increased with increasing the concentration of colloidal nanoparticle is an indication of antiviral properties of silver nanoparticles.

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