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ESTIMATION OF SLIVER NANOPARTICLES TOXICITY ON HUMAN GUT MICRO FLORA

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ABSTRACT

We synthesized silver nanoparticles (AgNPs) by using the biological material, the extract of *Allium sativum* (Garlic) and chemically synthesized AgNPs by using silver nitrate. The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 2 hours after diluting a small aliquot of the sample into distilled water. Toxicity of AgNPs was tested using toxtrak test in which, fresh overnight broths of *Bacillus subtilis* and resazurin dye was used to calculate percentage inhibition (PI). The PI is a relative measure only and since there is toxic substances that increase respiration, to give result in a negative number. The PI of both chemically and biologically synthesized AgNPs was compared in order to evaluate toxic effect value. The toxic effect value PI of chemically synthesized AgNPs is much greater (85.45%) than the biologically synthesized AgNPs from Garlic (46.35%). These observation shows that the bacteria *Bacillus subtilis* killed by chemically synthesized AgNPs are more as compare to biologically synthesized AgNPs.

INTRODUCTION

Toxicity is the degree to which a substance can damage an organism. Toxicity can refer to the effect on a whole organism, such as an animal, bacterium, or plant, as well as the effect on a substructure of the organism, such as a cell (cytotoxicity) or an organ such as the liver (hepatotoxicity). Toxicity is mainly dose dependent thus drugs should have more bioavailability and reduced toxic effects on cellular environment and normal micro flora inside the human body. Silver nanoparticles has significant role in the field of diagnostic (Schultz *et al*., 2000), antimicrobial and therapeutics (Rai *et al*., 2009; Elechiguerra *et al*., 2005). The silver ion (Ag+) is bioactive and in sufficient concentration readily kills bacteria in vitro. Silver also kills bacteria in external wounds in living tissue, so physicians use wound dressings containing silver sulfadiazine (Ag-SD) or silver nanomaterials to treat external infections (Qin *et al*., 2005; Hermans *et al*., 2006). The disinfectant properties of silver are used in medical applications, such as urinary catheters and endotracheal breathing tubes in reducing incidences of catheter-related urinary tract infections and ventilator-associated pneumonia, respectively (Saint *et al*., 1998; Kollef *et al*., 2008). Toxicity caused due to increased dosage of silver nanoparticles may cause accumulation of silver or silver sulfide particles in the hair, skin, kidneys, liver, heart and serious neurologic, renal, or hepatic complications, as well as headaches, stomach distress, fatigue, and skin irritation have been reported (Lansdown *et al*., 2006; Brandt *et al*., 2005; Stepien *et al*., 2009). Because of such a wide range of applications, numerous methods concerning the fabrication of silver nanoparticles, as well as various silver-based compounds containing ionic silver (Ag+) or metallic silver (Ag0) have been developed (Lu Y *et al*., 2007; Wilmer *et al*., 2006; Mallikarjuna NN *et al*., 2007; Chen M *et al*., 2006; Lou *et al*., 2006). Among the synthetic methods used for the preparation of silver nanoparticles, some toxic chemical used as a reducing agent such as NaBH4, citrate, or ascorbate is most commonly used (Kuo PL *et al*., 2003; Gardea-Torresdey *et al*., 2002; Gardea-Torresdey *et al*., 2003). Considering that such reducing agents may be associated with environmental toxicity or biological hazards, the development of a green synthesis for silver nanoparticles is desired. Biosynthesis of nanoparticles using plant extracts is the favourite method of green, eco-friendly production of nanoparticles and exploited to a vast extent because the plants are widely distributed,

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easily available, safe to handle and with a range of metabolites. The plant material used for biosynthesis of nanoparticles includes Angiospermic plants such as Helianthus annus, Oryza sativa, Zea mays, Sorghum bicolour (Arangasamy Leela *et al*., 2008), Eucalyptus hybrida (Manish Dubey *et al*., 2009), Artocarpus heterophyllus (Thirumurugan *et al*., 2010) and Gymnospermic plants such as Cycas (Anal K. Jhaa *et al*., 2010) and many more. Mainly our study is based on evaluating the percentage toxicity of chemically synthesized silver nanoparticles and biologically synthesized silver nanoparticles from garlic. Biologically synthesized silver nanoparticles are biocompatible and friendlier to the nominal human body micro flora and do not severely disturbs it during the ingestion of drugs containing silver nanoparticles. Normal gut micro flora of a human body contains *Bacillus subtilis* which is a Gram-positive, catalase-positive bacterium. It is mainly present in the normal gut flora of humans and used as a probiotic in healthy individuals which rarely causes food poisoning. It has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions but still silver nanoparticles prove to be toxic and killing most of them. As a result of the abundant and desirable medicinal.

Characteristics of garlic (R. Naganawa *et al*., 1996; P. Tedeschi *et al*., 2007; M. S. Rahman *et al*., 2007; K. Srinivasan *et al*., 2005), these nanoparticles may be applicable in biomedical therapies, diagnosis, and sensing or aid in the development of novel technologies with significant attention to nanoparticle health and safety. The present study is based on evaluating the percentage toxicity of chemically synthesized silver nanoparticles and biologically synthesized silver nanoparticles from Garlic. Biologically synthesized AgNPs are biocompatible and friendlier to the nominal human body micro flora and do not severely disturbs it during the ingestion of drugs containing AgNPs. Normal gut micro flora of a human body contains *Bacillus subtilis* which is a Gram-positive, catalase-positive bacterium. It is mainly present in the normal gut flora of humans and used as a probiotic in healthy individuals which rarely causes food poisoning. It has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions but still AgNPs prove to be toxic and killing most of them.

MATERIALS AND METHOD

Materials

Sample (Garlic) for the biological synthesis of AgNPs were collected from local market of Meerut in April 2012. Silver nitrate ($AgNO_3$) and Tri sodium citrate ($Na_3C_6H_5O_7.2H_2O$) of analytical grade for chemical synthesis of silver nanoparticles were obtained from the laboratory. A stock of 1mM was prepared and stored in a brown bottle to avoid light disintegration of Silver nitrate and a stock of 38mM of Tri sodium citrate was prepared.

Methods

Sample collection

Peel off Garlic (*Allium sativum*). The plant material was ground in blender and extracted in distilled water.

Collection of pathogens

The cultures of *Bacillus subtilis* used for demonstrating the toxic effects of silver nanoparticles were collected from the Biotechnology laboratory of Meerut Institute of Engineering & Technology, Meerut.

Preparation of the biologically synthesized silver nanoparticles

100gm of Garlic was ground to obtain the extract. Extract was filtered using Whatmann No-1 filter paper and filtrate was collected and centrifuged at 5000 rpm for 20 min. Silver nitrate was used as precursor for synthesis of AgNPs. 5ml of 1mM silver nitrate aqueous solution was added to 100ml of clear plant extract (Supernatant). Then the conical flask containing the solution was put into a shaker (150 rpm) at 30°C for 72 hrs. In this process the *Allium sativum* (Garlic) extract acts as the reducing and stabilizing agent. AgNPs were obtained gradually by the erosion and chemical degradation of plant extract.

Preparation of chemically synthesized Silver nanoparticles

38mm Tri sodium citrate ($Na_3C_6H_5O_7.2H_2O$) and 1mm Silver nitrate $AgNO_3$ were used for chemically synthesized AgNPs. Take 50 ml aqueous silver nitrate and boiled up to 70-80 ^{0}C and mix to 10 ml Tri sodium citrate drop by drop wise method. The solution was continuously stirred through magnetic stirrer for 4-5 min. After proper mixing the solution was then incubated at 30°C for 45 min. AgNPs were obtained gradually by the erosion and chemical degradation.

Characterization

The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 2 hours after diluting a small aliquot of the sample into distilled water. Technique involves ultra-violet and visible spectroscopy UV-Visible absorption spectra were measured using UV-Vis spectrophotometer operated at a resolution of 1 nm. The colour change in reaction mixture solution was recorded through visual observation which showed bio reduction of silver ions in aqueous solution. UV-Vis spectral analysis was done by using UV-VIS spectrophotometer V-530 (JASCO).

Toxtrak test

3 test tubes of 48 hrs broths of *Bacillus subtilis* were used for Toxtrak test for determining toxicity of chemically and biologically synthesized AgNPs against human gut micro flora (*Bacillus subtilis*). One is marked as control and other two are incubated with 1 ml chemically synthesized AgNPs and 1 ml garlic AgNPs respectively for 4 hrs. The concentration of the AgNPs ranges from 25-50µg/ml in both the solutions. Dye is added in the volume of 40µl per test tube and incubated. An initial absorption just after adding the dye is recorded from every test tube and then the absorption is taken after every 1 hr interval for 4hrs.

RESULTS AND DISCUSSIONS

The plant extract from garlic supernatants were pale yellow before the addition of silver ions and this changed to a brownish color on completion of the reaction with ions. This clearly indicated that the reduction of the ions occurs

extracellularly through reducing agents released in to the solution by garlic and was analyzed by the UV-Vis spectra. A surface plasmon peak located at 399nm (2.2080) was observed for the AgNPs synthesized from garlic extract (Figure1). Similar discussion of results of UV Visible spectrophotometry producing max peak 399 nm for garlic have been made by scientists (N.C..J. Packia Lekshmi *et al*., 2012). In case of chemically synthesized AgNPs the solution turns transparent golden brownish which shows the presence of AgNPs and on performing UV-Vis spectrophotometry the absorption peak is observed to be at 400 nm.

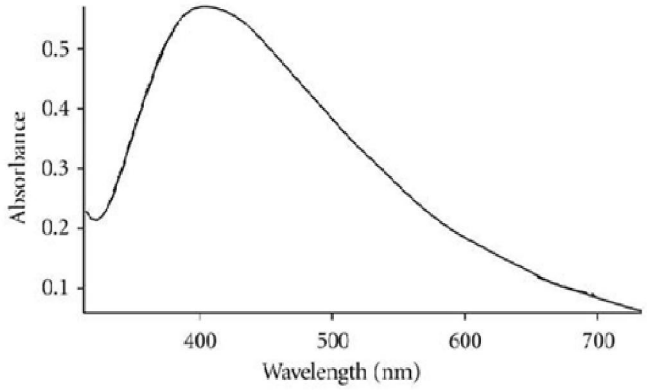

Figure 1. UV Visible graph of silver nanoparticles synthesized from garlic extract

Toxtrak test

In the presence of toxicity, the decreasing rate of degradation also decreases the reduction of resazurin and these changes are measured by the changes in absorbance of the sample compared to a control. The absorbance test is carried out at a wavelength of 603 nm, which is specific for the blue color. The inhibition is expressed as follows:

%I= [1- (ΔAs / ΔAc)] *100

Where ΔAs and ΔAc represent the change (decrease) in absorbance for the sample and the control, respectively. In this case, Δ is the initial-final value. The percent inhibition (% I) is a relative measure only. Since there are toxic substances that increase respiration, the % I can result in a negative number. Absorption of control and various broths treated with chemically A_{CS} and biologically A_S synthesized AgNPs (Table1). To determine % I value First we are calculate changes/differences (decrease) in absorbance for the control (ΔAc), chemically synthesized AgNPs (ΔAcs) and biologically synthesized AgNPs (ΔA_S) than put the value of decrease in % I equation to get finally toxicity percentage.

Control sample

ΔAc= 0.2163-2.9314
= -2.7151

Toxicity of chemically synthesized AgNPs

ΔAcs=1.5962-1.9914
= -0.3952
%I= [1- (ΔAs / ΔAc)] * 100
= [1-(-0.3952/-2.7151)]*10
= 85.45%

Toxicity of biologically synthesized AgNPs from garlic

ΔAs= 1.6401-3.0969
= -1.4568
%I= [1- (ΔAs / ΔAc)] * 100
= [1-(-1.4568/-2.7151)]*100
= 46.35%

The above data clearly indicates that the toxic effect of chemically synthesized AgNPs is much greater than that of biologically synthesized AgNPs. Toxicity of chemically synthesized AgNPs is 85.45% which is a lot of high in comparison to 46.35% toxicity of garlic AgNPs. The results of previously published work in which the toxicity of biologically synthesized AgNPs from onion extract against bacteria are 51.39% which, is very less as compare to chemically synthesized AgNPs (Tyagi *et al*, 2013). These observation shows that the bacteria killed by chemically synthesized AgNPs are large and thus degradation of dye is less when compared to biologically synthesize one. Thus AgNPs that are synthesized using plant extracts are proved to be more biocompatible and less toxic to cellular microenvironment and normal gut micro flora inside the human body. This study relates that the drugs using AgNPs should utilize biosynthesized AgNPs reducing the risk of toxicity.

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Table 1. Absorption readings of control, various broths treated with chemically and biologically synthesized silver nanoparticles

INCUBATION PERIOD WITH DYE	TEST TUBE 1 (CONTROL)	TEST TUBE 2 (CHEMICALLY SYNTHESISED AgNPs)	TEST TUBE 3 (BIOLOGICALLY SYNTHESISED AgNPs)
0 HOUR	2.9314	1.9914	3.0969
1 HOUR	2.5528	1.8327	3.0000
2 HOURS	2.0489	1.7375	2.7447
3 HOURS	1.0041	1.6049	1.8794
4 HOURS	0.2163	1.5962	1.6401

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