

Facile fabrication and characterization of silver nanoparticles by sunn pest (*Eurygaster integriceps puton*) damaged wheat and evaluation of its antibacterial and cellular toxicity toward liver cancer cell lines

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Abstract

Background & Aims: Silver nanoparticles (AgNPs) are among the most explored nanomaterials and are widely employed in medical, pharmaceutical, and bioengineering application. The aim of this study was to investigate the antibacterial and cell toxicity effects of fabricated AgNPs by green method at room temperature.

Materials & Methods: In this work, AgNPs were fabricated by green method, which uses the seed extract of *Triticum aestivum* damaged by sunn pest (*Eurygaster integriceps* Puton) (SPDSPDWAgNPs) at room temperature. Powder X-ray Diffraction (PXRD) and Transmission Electron Microscopy (TEM) were used for characterization of synthesized nanoparticles. Disk diffusion method and MTT analysis were used for antibacterial and cell toxicity effects, respectively.

Results: The synthesis of SPDSPDWAgNPs was relevant by color change of solution, turning from light-yellow to dark-brown, respectively. α -glycosidase, protease and α -amylase are some identified enzymes of salivary glands of sunn pest which can affect the synthesis of nanoparticles. The results showed that SPDSPDWAgNPs have spherical shapes and had a different range from 1 to 25 nm. Moreover, the biosynthesized SPDSPDWAgNPs have high antibacterial properties toward the most common inflectional bacteria. Furthermore, the cytotoxicity results show the fabricated SPDSPDWAgNPs prevent the growth of cancer cells.

Conclusion: We concluded that this production manner of nanoparticles could be employed for large-scale synthesis of AgNPs, pharmaceutical, and antiseptic uses.

Keywords: Silver nanoparticle, Anticancer, Antimicrobial, Green chemistry.

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Introduction

Silver nanoparticles (AgNPs) have lately gained popularity for an extensive variety of biological uses due to their increased and exclusive biochemical activities

(1, 2). The performance of silver nanorates in-fighting and eliminating pathogenic agents is well known due to its interaction with the surface of the cell membrane of microbes, and research in this field is still ongoing (3-

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5). The Ag ions reduce sheath penetrability, causing the death of microbes (6). Investigations displays that AgNPs activity against microbes are more effective than Ag in their bulk (7-9). In recent years, AgNPs have gained substantial consideration and are widespread considered nanostructures (10). Because of distinctive physical and biochemical activities, they have enormous applications in medicine, pharmaceutical industries, horticulture, and catalysis (11, 12). There are various reports that show that silver nanoparticles are made in different ways and each of the final products has its own properties (13-15).

Physicochemical ways are being used for AgNPs fabrication (16, 17). These ways have their specific benefits and difficulties. Physical ways need for example high pressure and therefore particular tools are required (17, 18). Chemical ways have the advantage that the size of nanoparticles can be controlled but leads to non-ecofriendly and toxic products (19-21). Biological ways of AgNPs fabrication do not need the employ of toxic materials and act as an ecofriendly way (22-24). Green synthesis ways have attracted many considerations in the recent years due to the employ of safe materials, and permit the production of ecofriendly materials with least toxic effects (25-27). These ways typically use natural-based materials to fabrication of nanostructures (28). Plants-mediated ways are known as one of the safe and reliable ways in the synthesis of nanoparticles (29-31). Nevertheless, it is beneficial to employ plants for fabrication of nanoparticles as it does not need specialized tools (32). Various parts of a plant such as fruits, leaf or stem can participate in the synthesis of nanoparticles (33, 34). There are important and valuable secondary metabolites such as phenols and flavonoids in plants, which can play a critical role in the synthesis and improvement of the quality of nanoparticles (35-37). Wheat is one of the important grains all over the world, which is abundantly found

everywhere and can be used as a reliable and affordable source in the synthesis of various nanoparticles.

The purpose of this work was to examine the fabrication of silver nanoparticles by *Triticum aestivum* damaged by sunn pest (*Eurygaster integriceps Puton*) (SPDWAgNPs) as they organize a resource of materials, which makes them candidates for fabricating nanoparticles without the requirement addition of any things to the fabrication.

Materials & Methods

Wheat seeds as prepared from research and innovation Agriculture Institute of Mashhad, Iran. AgNO₃ with purity of 99% was obtained from Sharlu-Spain. Other materials were obtained from Merck. The biosynthesized silver nanoparticles were fully characterized by transmission electron microscopy (TEM) and powder X-ray diffraction (PXRD) techniques.

Preparation of Wheat Extract:

Wheat grains were first powdered with a mill, and then 3 grams of powder and mixed with 300 grams of deionized water and stirred for 24 hours. The mixture was filtered with a filter paper and the resulting solution were stored in the refrigerator at a temperature of 5 °C for later use. The final solution as a reducing factor is employed for the fabrication of nanoparticles.

Biosynthesis and Characterization of Silver Nanoparticles:

AgNO₃ was selected as the initiator for the fabrication of nanoparticles. The AgNPs were made using the reaction of wheat solution and one mM solution of silver nitrate. 1 mM Silver nitrate solution was combined with the solution of wheat at a ratio of 90:10 (v/v). After that, the solution was placed on the stirrer for 48 hours. The biosynthesized colloidal AgNPs

were characterized using X-ray diffraction (XRD) analysis and Transmission Electron Microscopy (TEM).

Antibacterial Activity of AgNPs:

The disk diffusion technique was applied to assess the bactericidal properties of SPDWAgNPs towards *Bacillus subtilis* as a Gram-positive bacterium and *Escherichia coli* as a Gram-negative bacterium. The bacterial culture was regulated to 1.5×10^8 colonies. The surface of agar was injected three times by bacterial suspension. At that time, disks soaking in SPDWAgNPs solution, were placed on the agars. The extract was used as negative control. The plates were kept in the incubator for 24 seconds. Gentamicin and streptomycin were employed as positive control in the antibacterial tests.

Cell Toxicity Properties Against Liver Cancer Cell Lines:

The cell toxicity of biosynthesized SPDWAgNPs was measured against liver cancerous cell lines (HepG2) by MTT method. HepG2 cell line was obtained from the medicinal school of Mashhad, Iran. The cells which were established in the DMEM and FBS. The medium was adjusted to comprise 1% penicillin, streptomycin and l-glutamine. Diverse treatments (e.g., 0, 0.5, 1, 2, 4, 8, 15, 30, 60, 125, and 250 $\mu\text{g/mL}$) of SPDWAgNPs were tested in bactericidal evaluation.

Results

Biofabrication of SPDWAgNPs:

The SPDWAgNPs biofabrication reaction has speciously been persuaded after the seeds parts of the extract have been added into the one mM AgNO_3 solution. Following to spending 48h in 25 °C, the color of the combination reaction was perceived to alteration into a dark brown colored, which verified the biofabrication of SPDWAgNPs (Figure 1). This change

of color has occurred because of the presence of the biomolecules in the extract which lead to the reduction of Ag ions into SPDWAgNPs.

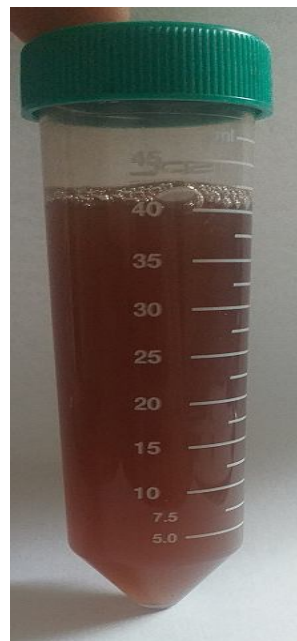


Fig 1. Formation of biosynthesized silver nanoparticles using wheat extract.

Characterization of Ag-NPs:

The explained PXRD design in **Figure 2** displays the specific peaks at 32.19°, 38.15°, 44.28°, 64.46° in regard to the biofabricated SPDWAgNPs from the extract of wheat seeds, respectively. This data confirms the preparation of face-centered cubic (FCC) crystalline elemental silver that is in agree with pervious published resources (38).

Transmission electron microscopy (TEM) has been utilized for identifying the form, dimension, and morphology of nanoparticles. It has been detected that the SPDWAgNPs are actually well dispersed and contain sphere shaped particles, as it is revealed in **Figure 3**. The variety of particles size has been dignified to be in 1 25 nm.

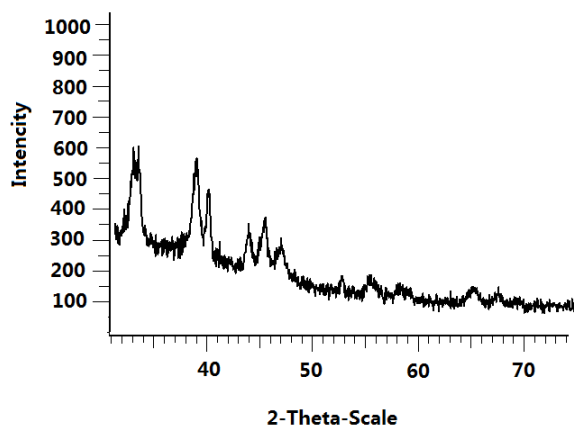


Fig 2. The PXRD pattern of biosynthesized AgNPs.

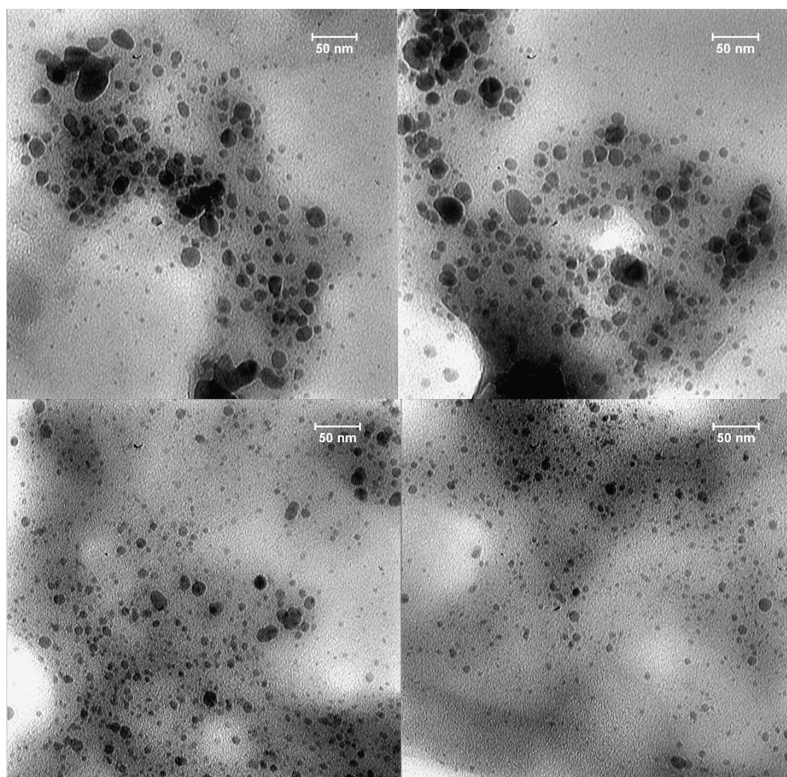


Fig 3. TEM images of biosynthesized AgNPs.

Bactericidal Activity of Biosynthesized AgNPs:

There is a report that shows, the AgNPs strongly inhibited the bacterial growth and propose using of Ag-coated surfaces can release antibacterial factors (39). As

a result of their bactericidal properties, AgNPs have been used generally in the numerous arenas of food, pharmaceutical industries, and ecological uses (40). These specific NPs have been tested for their bactericidal activities toward both gram positive

(*B.subtilis*) and gram negative (*E.coli*) bacteria displaying the areas of reticence. Based on the IZ (Inhibition Zone) created biofabricated AgNPs, the accommodation of great antibacterial properties has been detected toward all of the identified infective bacteria. The control treatment was not able to show any bactericidal properties. The calculation results in the case of antibacterial properties of synthesized AgNPs, which have been obtained from the disc diffusion technique, are specified and existing in Figure 4 while the IZ of antibacterial has been revealed in Table 1. The AgNPs have shown an effectual antimicrobial property that is purportedly a result of the massive surface area, which offers an improved interaction with the cell wall

of bacteria. In the existence of oxygen, the redox reaction produces OH radicals that oxidase could be employed as an antimicrobial factor. The contact of bacteria and nanomaterial surface at the interface is multifaceted and is influenced by the surface assets of nanomatter, the adjacent environment and the kind of bacteria. The mechanism of the bactericidal influence of Ag is usually supposed to include the contact of Ag-ions with (-) charged types existent in the cytoplasm membrane. It is also suggested that the decrease of cytoplasmic membrane or segregate from the cell wall cause to condensed-DNA with subsequent damage in capability to multiply on being infiltrated using Ag-ions.



Fig 4. Antibacterial properties of biofabricated of AgNPs against *E. coli*. and *B. subtilis* (Gentamycin and extract used as control+ and control- respectively).

Table 1. Diameter zones of inhibition (mm) using AgNPs toward pathogenic bacteria.

Bacteria	Test (AgNPs)	Control negative	Control positive
		(Extract)	(Gentamycin)
<i>Bacillus subtilis</i>	15	0	26
<i>Escherichia coli</i>	12	0	19

Cytotoxicity activity:

Biocompatibility is related to the behavior of biological materials in diverse situations and refers to the ability of a material to perform with the appropriate host-response in a particular state and one of these important cases is cellular toxicology (41-44). The cell toxicity activity of the biofabricated SPDWAgNPs on cancer cells (HepG2) has been studied using the MTT analyze. The cell treatments have been done with a diverse dose of SPDWAgNPs (0, 0.5, 1, 2, 4, 8, 15, 30,

60, 125 and 250 μ g/mL). As it is shown in Figure 5, the MTT analyze has displayed a dose-dependent on the %viability of HepG2 cells. The cytotoxicity influences of SPDWAgNPs are perhaps as a result of the effectual contact of Ag-atoms with various bio-groups inside the cell. According to the results obtained in this research, it can be concluded that the use of green synthesized silver nanowires can be used as antipathogenic agents and also in the treatment of cancer.

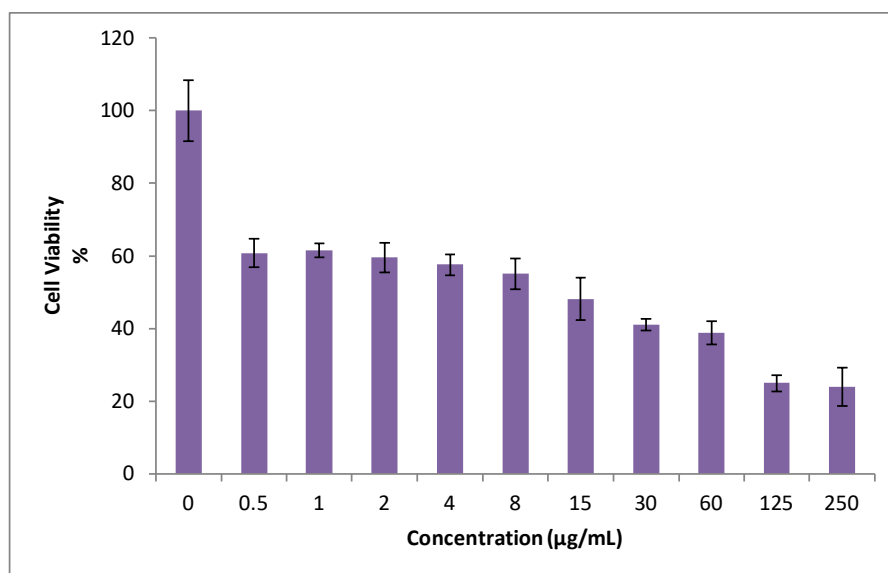


Fig 5. The cell toxicity results of green bio-fabricated SPDWAgNPs.

Discussion

Nanoscience and nanotechnology has grown rapidly (45). One of the most important branches of the nanoscience is nanomedicine (46, 47). Nanomedicine has a wide range of application from the diagnosis and treatment of modern diseases (48, 49). There are several ways to synthesis of nanoparticles but most of them are ineffective because of harmful substances used in the synthesis of nanoparticles, which ultimately have various side effects and the production of nanoparticles with these methods is either not cost-effective or has

negative effects on the environment (39, 50). The usefulness of metal and metal oxide nanoparticles, including silver and silver oxide nanoparticle, has long been recognized. Silver nanoparticles and silver-based nanocomposites have been used for a variety of uses in recent decades (51). Many investigations about the silver nanoparticles (AgNPs) proved their significance as effective antimicrobial factors toward several pathogenic microorganisms (52-54). The antimicrobial activities of AgNPs have been relevant in the medical area for regulating microbial infections. Furthermore, AgNPs have shown great potential in the treatment of

diverse types of tumors, most especially cancer cell lines. In the present research, the successful synthesis of silver nanoparticles using sunn pest damaged wheat was shown. The structures of nanoparticles were investigated using PXRD and TEM, and the results revealed spherical shape nanoparticles with a size of 1-25 nm. The synthesized nanoparticles showed a high ability to inhibit the growth of pathogenic bacteria. In a study, the effects of silver nanoparticles, which were made by the green method using *Premna integrifolia*, were tested on pathogenic bacteria (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*) and the results showed an inhibitory effect on bacteria, which is consistent with our results (55). In the study of the effect of silver nanoparticles synthesized by the green method, they were tested against *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Streptococcus pyogenes* (56). The results of antibacterial effects in this study are consistent with the findings of the present study. The cytotoxicity results of the present study showed the effect of synthesized nanoparticles on the destruction of cancer cells. In various studies, the effect of silver nanoparticles prepared by the green method on different cancer cells was tested, and all of them showed the toxicity effect of nanoparticles against A549 lung cancer cells (57), breast cancer cells (58), and liver cancer cells (59), which are consistent with the cytotoxicity results of the present study.

Studies have shown that plant-mediated synthesized nanoparticles increase ROS levels that cause cancer cell death (60-62). Although various studies have shown the involvement of ROS in causing toxicity in cancer cells, the exact mechanism of this process is still unclear. The results of the present work showed that the synthesized nanoparticles can be produced on a large scale and used in disinfection industries. Also, the studies conducted in the future and in vivo can determine the interactions of

nanoparticles with other biological molecules in the animal model and clarify other mechanisms.

Disclosure statement

No potential conflict of interest was stated by the authors.

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Conflict of interest

None of the authors have any interest that conflicts with this study.

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