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Research Article

Antibacterial, Cytotoxic, and Cellular Mechanisms of Green Synthesized Silver Nanoparticles against Some Cariogenic Bacteria (*Streptococcus mutans* and *Actinomyces viscosus*)

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Background. The present study focused on the green synthesis of silver nanoparticles (AgNPs) using the *Astragalus spinosus* Forssk. aqueous extract. In addition, we evaluated the antibacterial activity of AgNPs as well as some cellular mechanisms against *Actinomyces viscosus* and *Streptococcus mutans* as the most causative agents of tooth decay. *Methods*. In this study, AgNPs were green synthesized by the precipitation method based on the reduction of silver ions (AgNO3) by *A. spinosus* extract. Antibacterial effects of the green synthesized AgNPs were performed by measuring the minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) through micro broth dilution method. In addition, we evaluated the reactive oxygen species (ROS) production, nucleic acid leakage, and protein leakage as the main antibacterial mechanisms of the green synthesized AgNPs against *A. viscosus* and *S. mutans*. The cytotoxicity effects of AgNPs against on human normal (NOF18 cells) and oral cancer (SCC4 cells) cell lines were also evaluated using MTT assay. *Results*. The green synthesized AgNPs have a spherical shape and are relatively uniform in size in the range of 30-40 nm. The MIC values for *S. mutans* and *A. viscosus* of the green synthesized AgNPs were 10.6 and 13.3 μg/ml, respectively, whereas the MBC values for *S. mutans* and *A. viscosus* of the green synthesized AgNPs were 21.3 and 26.6 μg/ml. The findings exhibited that ROS production, nucleic acid leakage, and protein leakage were increased after treatment of *A. viscosus* and *S. mutans* by the green synthesized AgNPs. The results demonstrated that the 50% inhibitory concertation (IC_{50}) values of AgNPs on NOF18 and SCC4 cells were 93.3 μg/ml and 41.2 μg/ml, respectively. *Conclusion*. Overall, the results of this study showed that *A. spinosus* extract has a good ability to produce silver nanoparticles. The AgNPs produced have significant antibacterial effects against some tooth decay bacteria. Our results also revealed that the green synthesized AgNPs are more cytotoxic against cancerous cell line than normal cell line. Further *in vivo* studies are required to investigate the side effects and to evaluate the effectiveness of these bacteria.

1. Introduction

Dental caries as a permanent damage in the enamel or hard surface of the teeth is a chronic microbial disease affecting humans worldwide [1]. Based on the World Health Organization (WHO) reports, the frequency of dental caries varies ranging from 60 to 80% in children and nearly 100% in adult people [2]. The oral cavity is an exceptional ecological site

Table 1: The minimum inhibitory concentration (MIC) and the minimum bactericidal concentrations (MBC) of the green synthesized AgNPs by *A. spinosus* extract on *S. mutans* and *A. viscosus*.

Drug	*A. viscosus*		*S. mutans*	
	MIC (μg/ml)	MBC (μg/ml)	MIC (μg/ml)	MBC (μg/ml)
AgNPs	13.33 ± 4.6	26.66 ± 4.6	10.6 ± 4.6	21.33 ± 1.15
Chlorhexidine	10.6 ± 4.6	21.33 ± 1.15	8.0 ± 0.0	16.0 ± 0.0

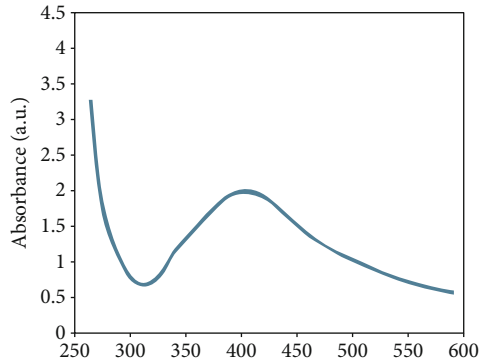

Figure 1: Ultraviolet absorption by silver nanoparticle green synthesized from the *A. spinosus* extract.

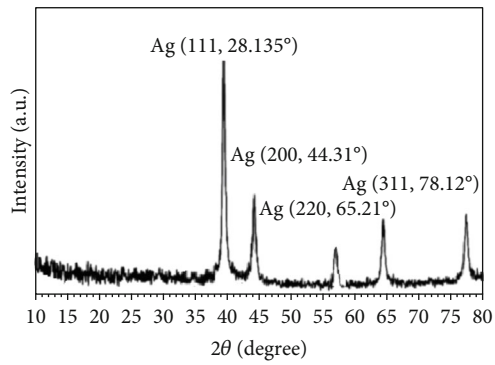

Figure 2: X-ray diffraction (XRD) analysis of the green synthesized AgNPs.

for microorganisms, where most of these microorganisms multiply on tooth surfaces and cause dental plaque (oral biofilm) [3]. The cariogenic bacteria are among the oral microbiota that ferment carbohydrates and subsequently produce acids and demineralize the tooth surfaces. Such bacteria, including *Streptococcus* spp., *Actinomyces* spp., and *Nocardia* spp., are well-known as the main cariogenic causes involved in the progress of tooth caries [3, 4]. Because of the available systemic antibiotics are not effective for treating oral bacteria or not specific to treat oral diseases, several antimicrobial agents such as chlorhexidine, fluoride, and quaternary ammonium salts have been used to target oral bacteria that cause oral diseases such as tooth decay [5].

Today, metal nanoparticles are used as attractive candidates to deliver many small drug molecules or large biomolecules [6]. Metal nanoparticles are widely used as important products in nanomedicine due to their unique physical properties [7]. Recently, studies have demonstrated that the chemical and physical techniques used to synthesize nanoparticles are often very expensive, and the presence of toxic and sometimes carcinogenic residues that produced these techniques usually results in harming effects of the nanoparticles [8]. Therefore, the development of reliable, nontoxic, and cost-effective methods for the synthesis of nanoparticles using plants (green synthesis) and microorganisms is highly valuable. Today, the use of plants as a renewable and inexpensive source for the synthesis of green nanoparticles has received much attention [9].

The existence of the secondary metabolites (e.g., polyphenols, alkaloids, terpenoids, quinones, and tannins) in plants encourages ion bioremediation and synthesis of some metal nanoparticles [10]. Among metal nanoparticles, silver nanoparticles (AgNPs) have emerged as a powerful product in the field of nanotechnology [11]. These nanoparticles have received special attention over the past few years due to their proper conductivity, chemical stability, catalytic activity, and antimicrobial properties [12]. *Astragalus* plants belonging to the Fabaceae family (with more than 3000 species) are one of the most important plant species around the world [13]. In traditional medicine, plants of the genus *Astragalus* were used for treating diseases and illness conditions such as bronchitis, cough, stomach ulcer, hypertension, gynecological disorders, and diabetes [14]. In modern medicine, previous studies also demonstrated that plants of *Astragalus* genus have some pharmacological properties such as anti-inflammatory, antidiabetic, anticancer, antioxidant, analgesic, and antioxidant [15].

The present study is aimed at green synthesis and characterization of the AgNPs using the *Astragalus spinosus* Forssk. aqueous extract. The study is also targeting the evaluation of the antibacterial activity of the AgNPs as well as some cellular mechanisms against *Actinomyces viscosus* and *Streptococcus mutans* as the most common agents in tooth decay.

2. Materials and Methods

2.1. Plant Materials and Extraction. Aerial parts of *A. spinosus* were obtained from country districts of Tabuk, Saudi Arabia, in April 2020. The collected herbs were then recognized by a botanist, and a sample of voucher of the herb was archived at the herbarium of College of Applied Medical Sciences, Al-Quwayiyah, Shaqra University, Saudi Arabia, for further experiments. Air dried fruits of *A. spinosus* (200 g) were extracted through percolation process with water consecutively for 3 days at 21°C. In the next step, the extracts were filtered by using filter paper (Sigma, Germany) and lastly evaporated in vacuum at 55°C by means of a rotary evaporator and preserved at −20°C until examining.

2.2. Green Synthesis of AgNPs. In this study, AgNPs were green synthesized by the precipitation method based on the reduction of silver ions ($AgNO_3$) through *A. spinosus* extract according to the method described by Sulaiman et al. [16]. To do this, 10 ml of the extract was added to

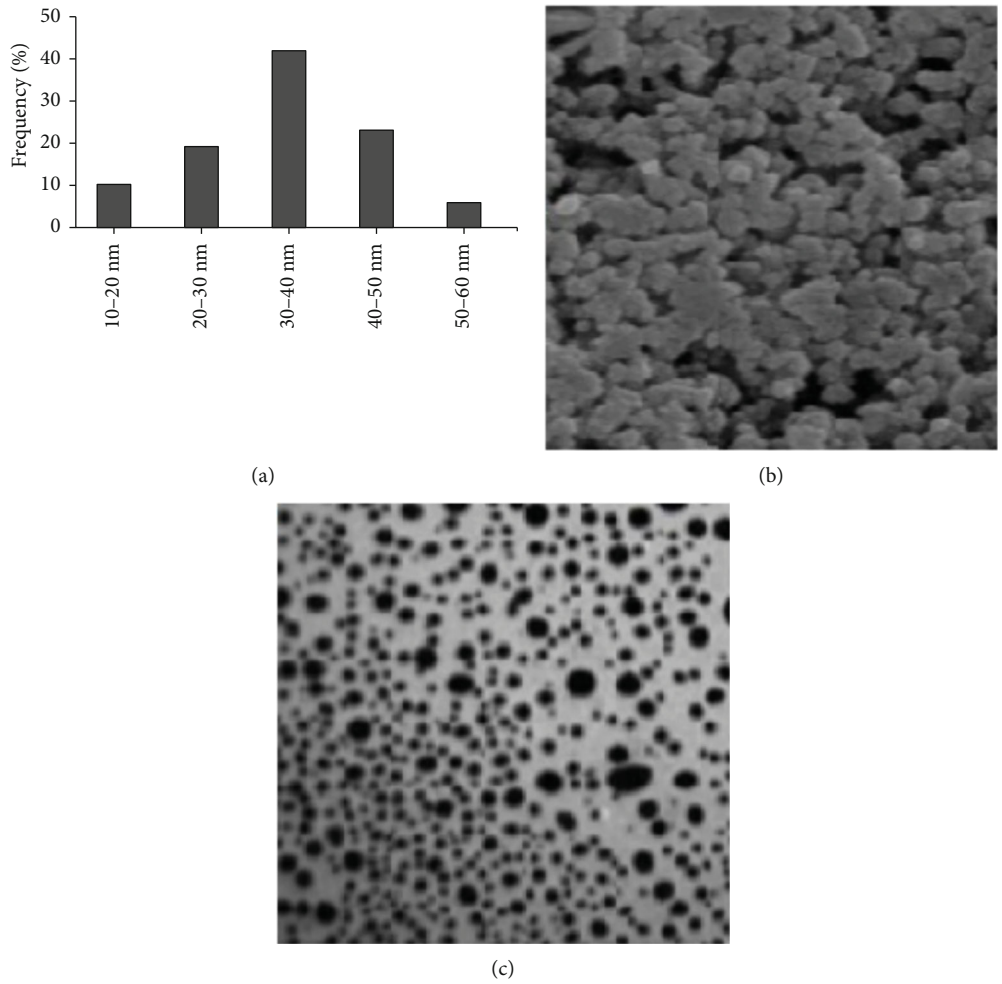

Figure 3: (a) The size distribution of the green synthesized AgNPs by *Astragalus spinosus* extract; (b) scanning electron microscope (SEM) and (c) transmission electron microscopy (c) of the green synthesized AgNPs displayed a spherical shape and are relatively uniform in size in the range of 30-40 nm.

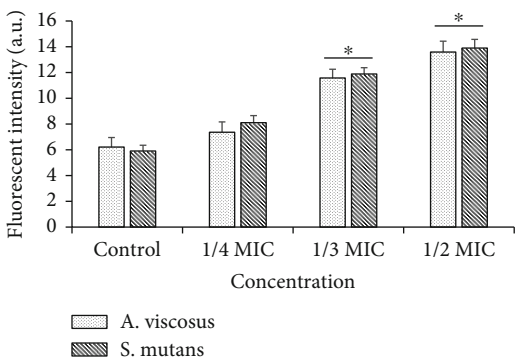

Figure 4: The reactive oxygen species (ROS) production of AgNPs on *S. mutans* and *A. viscosus*. Data are presented as Mean ± SD. * $p < 0.001$.

90 ml of $AgNO_3$ (1 Mm, Merck, Germany) and kept at room temperature and in the dark overnight to reduce silver ions and to reduce ions. The change in color of the extract from pale yellow to dark brown to black indicates the production of $AgNO_3$. In order to observe the color changes on the absorbance of the solution, a spectrophotometer in the range of 300-700 nm was investigated. The solution containing the nanoparticles was centrifuged at 12000 rpm for 15 minutes, and then, the supernatant was discarded.

2.3. UV-vis Spectroscopy Analysis. Surface plasmon resonance (SPR) of synthesized AgNPs was determined by using UV–vis spectrophotometer to approve the transformation of the Ag ions to AgNPs. Accordingly, 0.3 ml of the NPS solution was diluted with 3 ml of normal saline and was examined by UV–vis spectrum analysis employing a spectrophotometer device (Shimadzu UV2550, Japan) in the range of 300–700 nm.

2.4. X-Ray Diffraction (XRD) Analysis. XRD analysis was used to investigate the presence of nanoparticles by *A. spinosus* extract. In fact, this method examines the stepwise formation of biodegradable nanoparticles. The crystal structure of the synthesized nanoparticles was investigated by considering the Ka ray source of a copper lamp with a

Table 2: Some recent studies and their research findings on the green synthesis of silver nanoparticles (AgNPs) using various plants.

Authors	Years	Plant name	Results	Ref.
Emrani et al.	2018	*Glycyrrhiza glabra* and *Mentha piperata*	MIC values for AgNPs synthesized with *G. glabra* extract against Streptococcus mutans, Actinomyces viscosus, and Lactobacillus rhamnosus were 1.6, 6.25, and 50 mg/ml, and MIC for AgNPs synthesized with Mentha piperata extract against these bacteria were determined to be 12.5, 12.5, and 200 mg/ml, respectively	[29]
Majeed et al.	2016	*Salix alba*	These synthesized silver nanoparticles showed a good antibacterial activity against the bacteria isolates (*Lactobacillus* sp., *Streptococcus* sp., and *Staphylococcus* sp.)	[30]
Suwan et al.	2018	*Oryza sativa* L.	Antimicrobial test showed that the AgNPs obtained from green synthesis mediated by rice extracts have great antimicrobial activity against *Streptococcus mutans*	[23]
Hernández-Gómora et al.	2017	*Heterotheca inuloides*	AgNPs exhibited antibacterial activity against *Streptococcus mutans*, *Lactobacillus casei*, *Staphylococcus aureus*, and *Escherichia coli*	[31]
Tolouietabar et al.	2017	*Scrophularia striata*	AgNPs at the concentration of 5 mM showed significant antibacterial activity against *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Bacillus cereus*	[32]

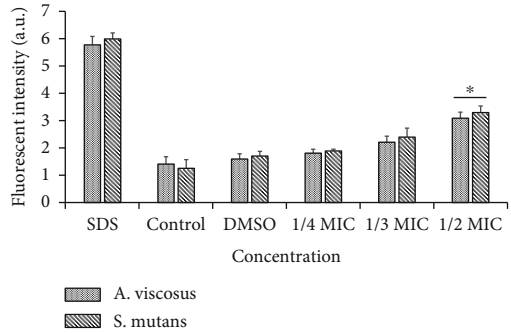

Figure 5: Effects of the green synthesized AgNPs on nucleic acid leakage from *S. mutans* and *A. viscosus* cells at 1/2 MIC, MIC, and 2 MIC. Data are presented as Mean ± SD. $^{*}p < 0.001$.

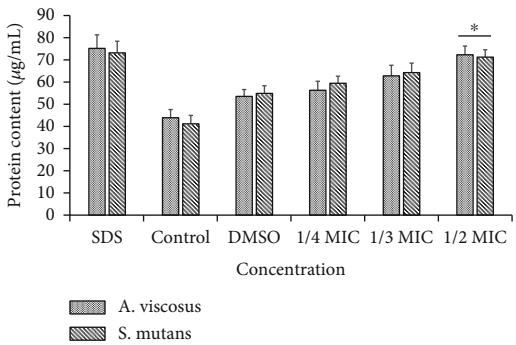

Figure 6: Effects of the green synthesized AgNPs on protein leakage from *S. mutans* and *A. viscosus* cells at 1/2 MIC, MIC, and 2 MIC. Data are presented as Mean ± SD. $^{*}p < 0.001$.

wavelength of X beams in $\lambda = 1.54\,A^0$ by a XRD device model 2000 APD (Italy).

2.5. Dynamic Light Scattering (DLS) and Electron Microscope. The specifications of synthesized AgNPs such as morphology (the size and shape) were studied by scanning electron microscope (SEM) (Mira3, made in Czech) with 15 kV, magnification of 10x, and resolution of 1 nm as well as transmission electron microscopy (TEM, Jeol JEM-1220, JEOL, Japan). In this study, the particle size was determined through DLS using the Zetasizer (UK, Malvern) device.

2.6. Antibacterial Effect of NPs

2.6.1. Bacteria. Each of the bacterial species of *S. mutans* (ATCC 35668) and *A. viscosus* (PTCC 1202) was cultured in Tryptic Soy Broth (TSB) Agar, Brain Heart Infusion (BHI, Difco, USA), and Mitis Salivarius Agar (MSA) at 37°C in an atmosphere containing 5% CO_2. Finally, growing bacteria were confirmed by means of Gram staining, catalase test, optochin, and bacitracin tests.

2.6.2. Preparation of Standard McFarland 0.5 Solution. To prepare the standard McFarland 0.5 solution, 0.5 ml of $BaCl_2$ (0.048 mol/l) ($2H_2O$ *w/v* $BaCl_{20}$ 1/175%) was added to 99.5 ml of sulfuric acid (0.18 mol/l) (*v/v* 1%). The suspension was stirred constantly, and the standard optical density was evaluated by absorbance measurement by means of a spectrophotometer at an optical length of 1 cm. The absorbance of 625 nm should be between 0.8 and 0.13 [17].

2.6.3. Preparation of 0.5 McFarland Solution of Studied Bacteria. Some colonies of bacteria were dissolved in 1 cc of physiological serum. Turbidity of bacterial solution was compared with the standard 0.5 McFarland solution [17].

2.6.4. Micro Broth Dilution. The minimum inhibitory concentration (MIC) of green synthesized AgNPs against *S. mutans* and *A. viscosus* was evaluated by micro broth dilution method based on the Clinical and Laboratory Standards Institute (CLSI) orders [18]. Initially, a stock of synthesized AgNPs is prepared with normal saline solvent and sterile Müller-Hinton broth culture medium. Then, 50 microliters of sterile Müller Hinton broth culture medium was added to the third to twelfth rows. From the stock made, 100 microliters is added to the first and second rows, and the dilution operation is performed from the second to the tenth rows. In this way, from the second row, 50 microliters to the third row and from the third row 50 microliters to the fourth row and up to the tenth row will be diluted in the same way.

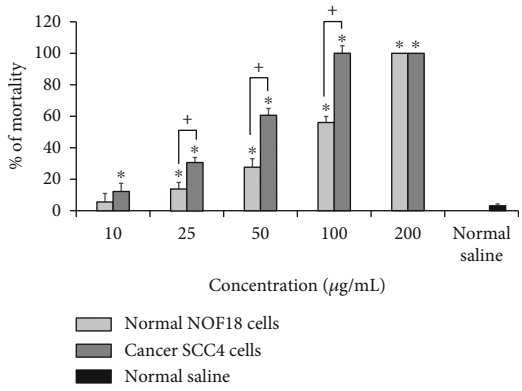

FIGURE 7: The cytotoxicity effects of the green synthesized AgNPs against human normal (NOF18 cells) and oral cancer (SCC4 cells) cell lines, which were evaluated using the colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Mean ± SD ($n = 3$). $^*p < 0.001$ difference was statistically significant compared with the control group (normal saline). $+p < 0.001$ difference was statistically significant.

Finally, after 24 hours of culture, the desired microorganism is added in the amount of 50 microliters equivalent to half McFarland turbidity (1.5×10^8 cfu/ml) to two to ten rows. The plates are placed in a shaker incubator for 24 hours at 37°C, and then, salts of 2, 3, and 5-triphenyltetrazolium chloride are used as a visual indicator for bacterial growth. Colorless wells are reported as MICs. The lowest concentration of the AgNPs in which no bacteria survived was considered to indicate the minimum bactericidal concentrations (MBC) of AgNPs. Normal saline is used for negative control, and chlorhexidine is used for positive control.

2.6.5. Analysis of the Reactive Oxygen Species (ROS) Generation. In the present investigation, we used 2′, 7′ -dichlorofluorescein diacetate (DCFH-DA, Sigma-Aldrich, Germany) to determine the level of bacterial ROS prompted by synthesized AgNPs. In summary, *S. mutans* and *A. viscosus* cells were separately incubated with 10 μM of DCFH-DA at 37°C for 30 min. Next, bacteria were separately treated with and without the green synthesized AgNPs at the concentrations of 1/2 MIC, 1/3 MIC, and 1/4 MIC for 3 h. Finally, the fluorescence intensity was measured at excitation/emission wavelength of 488/525 nm [19].

2.7. Effects of the Green Synthesized AgNPs on Nucleic Acid Leakage. In this study, we evaluated the effect of green synthesized AgNPs on the integrity of bacterial membranes, based on the methods described elsewhere [20]. Briefly, the bacteria suspension was treated with the green synthesized AgNPs at the concentrations of 1/4 MIC, 1/3 MIC, and 1/2 MIC and was incubated at 37°C for 30 min. In the next step, 1 ml of the bacteria was centrifuged for 1 minute at 10,000 rpm; the obtained residual sediment was washed with 1 ml normal saline, and then, the 3 μl of propidium iodide was added to the combination and kept for 10 minutes in the dark. Fluorescence was measured at excitation and emission wavelengths of 544 nm and 612 nm, respectively, using a ƒmax microplate spectrofluorometer (Molecular Devices, Sunnyvale, USA). The negative and positive controls were contained dimethyl sulfoxide (DMSO) and sodium dodecyl sulphate (SDS, 0.1%).

2.8. Effects of the Green Synthesized AgNPs on Protein Leakage. Here, we determined the effects of the green synthesized AgNPs on protein leakage based on the method explained by Du et al. [21]. In brief, the bacteria suspension was treated with the green synthesized AgNPs at the concentrations of 1/4 MIC, 1/3 MIC, and 1/2 MIC and was incubated at 37°C with shaking for 120 min. In the next step, after centrifuging the bacteria suspensions at 4000 rpm for 240 s, 0.05 ml of the suspensions supernatant was mixed with 0.95 ml of Bradford reagent. Finally, the protein content was assessed according to the Bradford's method [22]. The negative and positive controls were contained dimethyl sulfoxide (DMSO) and sodium dodecyl sulphate (SDS, 0.1%). The absorbance was measured at 590 nm using a microplate reader spectrophotometer (BioTek Winooski, VT, USA).

2.9. Cytotoxicity Effects. The cytotoxic effects of the green synthesized AgNPs against human oral squamous cell carcinoma cells (SCC4, American Type Culture Collection/ ATCC CRL-1624) and human normal oral fibroblasts (NOF18) were evaluated using the colorimetric MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) according to the method described elsewhere [23]. SCC4 and NOF cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) (UFC-Biotech, SA) supplemented with 15% (*v*/*v*) fetal bovine serum (FBS) (Thermo Fisher, USA), penicillin (100 IU/ml), and streptomycin (100 μg/ml) (UFC-Biotech, SA). Then, SCC4 and NOF18 cells (5×10^4/ml) were treated with the green synthesized AgNPs (10-200 μg/ml) for 48 h in microplates at 37°C with 5% CO_2 [24, 25].

2.10. Statistical Analysis. All statistical tests were carried out using the SPSS software version 25.0 (SPSS, Inc.). To compare the results among tested groups, we applied the unpaired sample *t*-test and one-way analysis of variance (ANOVA) and the Dunnett's test. $p < 0.05$ was measured statistically significant.

3. Results and Discussion

Nanoparticles have a major impact on all aspects of human life due to their special properties such as size, shape, and morphology, and among them, metal nanoparticles such as silver, gold, platinum, and palladium have many applications in various scientific fields such as medical engineering and health [26]. The synthesis of silver nanoparticles has long been widespread due to some of their biological properties such as anticancer, antibacterial, antioxidant, and other effects (12). Today, the use of plants as sustainable and available sources in the production of biocompatible nanoparticles has been considered by many researchers in recent years, and the advantages of this method include biocompatibility, cheapness, nontoxicity, and production of high purity nanoparticles [27]. It has already been proven that

compounds, secondary metabolites, and biomolecules such as carbohydrates, lipids, phenols, flavonoids, tannins, acids, resins, and terpenes in plants play an important role in reducing nanoparticle ions [10]. In recent decades, there has been a growing attention of research on the efficiency of green synthesized nanoparticles on numerous diseases, including microbial infections [6, 7], although various laboratory and experimental investigations have reported the promising antiparasitic effects of silver nanoparticle green synthesized using various natural resource against a wide range of Gram-negative and Gram-positive bacteria; however, their efficacy as well as their toxicity is still debatable and questionable (Table 1) [23, 28–32]. The present study is aimed at green synthesis and characterization of the AgNPs using the *A. spinosus* methanolic extract. In addition, we evaluated the antibacterial activity of the AgNPs as well as some cellular mechanisms against *A. viscosus* and *S. mutans* as the most common agents in tooth decay.

The obtained results demonstrated that complete reduction of Ag^+ ions to AgNPs was performed by changing the color of the culture medium and spectroscopy. The change in color of the sample to dark brown is a clear sign of the synthesis of silver nanoparticles. The presence of a peak at 413 nm by UV-vis spectroscopy confirmed the synthesis of AgNPs (Figure 1). Since the peak formed at a wavelength of 400 to 450 nm indicates the formation of silver nanoparticles and is related to the surface plasmon resonance of silver nanoparticles, which is attributed to the induction of free electrons in nanoparticles, Figure 2 shows the XRD analysis of AgNP green synthesized from the *A. spinosus* extract. The XRD pattern showed that peaks 111, 200, 220, and 311 at 28.135°, 44.31°, 65.21°, and 78.12° corresponded to nanocrystals and silver cubic structures. Nonappearance of other peaks confirmed the purity of AgNPs used in the analysis. The size distribution of the of the green synthesized AgNPs was in the range among 5-60 nm, while the most common particles of the green synthesized AgNPs had the size of 30-40 nm (Figure 3(a)). As shown in Figures 3(b) and 3(c), by TEM and SEM, the green synthesized AgNPs have a spherical shape and are relatively uniform in size in the range of 30-40 nm.

In order to measure the electrophoretic mobility and charge of each nanoparticle sample, we performed the zeta potential experiment, whereas the high zeta potential value exhibited a high electric charge on the surface of the NPs that indicates promising repellent forces among the particles, which inhibits aggregation and result in stabilizing the NPs in the medium. Based on the obtained results, in optimal conditions (pH = 7.2), the value for the zeta potential was -14.6 and width (mV) with 100% intensity and indicated that the synthesized AgNPs are stable because of the electrostatic repulsion without adding a different physical or chemical capping agent (Figure 4).

Considering the antibacterial effects of green synthesized AgNPs, as shown in Table 1, the MIC values of green synthesized AgNPs for *S. mutans* and *A. viscosus* were 10.6 and 13.3 μg/ml, respectively, whereas the MBC values for *S. mutans* and *A. viscosus* of the green synthesized AgNPs were 21.3 and 26.6 μg/ml, respectively. Previous studies demonstrated the antibacterial effects of AgNPs synthesized from a number of plants (e.g., *Theobroma cacao*, *Pteridium aquilinum*, *Aloe vera*, *Mangifera indica*, *Azadirachta indica*, *Solanum indicum*, and *Ziziphus xylopyrus*) against both Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Basillus* spp., *Escherichia coli*, *Salmonella typhi*, *Shigella flexneri*, and *Klebsiella pneumoniae* [33] (Table 2).

By DCFH-DA assay, we assessed that whether antibacterial effects of the green synthesized AgNPs may be related to ROS. As shown in Figure 4, although fluorescence intensity was increased in a dose-dependent manner, however, a significant increase ($p < 0.05$) was observed at the concentration of 1/3 MIC and 1/2 MIC. These findings indicated that the green synthesized AgNPs mediated ROS production compared with that of the control group. It has been proven that ROS prompted by nanoparticles can cause the disruption in biomolecules and organelle structures and result in protein oxidative carbonylation, peroxidation of lipids, DNA/RNA rupture, and membrane structure damage, which further result in cell death [34].

As exhibits in Figure 5, the membrane disruption of bacteria was observed by an increased uptake of propidium iodide by *S. mutans* and *A. viscosus* cells treated with the green synthesized AgNPs in a dose-dependent manner. A significant nucleic acid leakage ($p < 0.05$) from bacteria cells was observed after treatment with 1/2 MIC of AgNP when compared to the control, whereas at concentrations of 1/3 MIC and 1/4 MIC, the green synthesized AgNPs were not able to cause significant nucleic acid leakage from bacteria cells in comparison with the control. Figure 6 shows the protein content after exposure of *S. mutans* and *A. viscosus* with the green synthesized AgNPs at the concentrations of 1/2 MIC, 1/3 MIC, and 1/4 MIC. The results exhibited that the green synthesized AgNPs displayed significant ($p < 0.05$) protein leakage at 1/2 MIC. However, at concentrations of 1/3 MIC and 1/4 MIC, there was no significant protein leakage in comparison with the control untreated cells. In line with our results, Rajesh et al. have demonstrated that silver nanoparticles are able to increase the protein leakage through the membrane of *Klebsiella pneumoniae* [35]. In addition, Abbaszadegan et al. have revealed that AgNPs displayed its antibacterial effects against some Gram-positive and Gram-negative bacteria through the protein leakage from the cell [36]. Therefore, the findings of the present study exhibited that ROS production, nucleic acid leakage, and protein leakage are the main antibacterial mechanisms of the green synthesized AgNPs against *A. viscosus* and *S. mutans*.

Here, the cytotoxicity effects of the green synthesized AgNPs against on normal (NOF18 cells) and cancer (SCC4 cells) cell lines were evaluated using MTT assay via measuring their IC_{50} values. Figure 7 shows the cytotoxicity effects of the green synthesized AgNPs against on NOF18 and SCC4 cell lines 48 h. The results demonstrated that the IC_{50} of AgNP value for NOF18 and SCC4 cells were 93.3 μg/ml and 41.2 μg/ml, respectively. Previously, Khorrami et al. have evaluated the cytotoxicity effects of AgNPs synthesized from *Juglans regia* extract against L-929

fibroblast normal cells and MCF-7 cells [37]; in line with our results, they have reported that the green synthesized AgNPs are cytotoxic against cancerous cell line while being nontoxic for normal cell line.

4. Conclusion

Overall, the results of this study showed that *A. spinosus* extract has a good ability to produce silver nanoparticles. The AgNPs produced have significant antibacterial effects against some tooth decay bacteria. Our results also revealed that the green synthesized AgNPs are more cytotoxic against cancerous cell line than normal cell line. Further *in vivo* studies are required to investigate the side effects and to evaluate the effectiveness of these bacteria. Therefore, this promising silver nanoparticle green synthesized using the *A. spinosus* Forssk. aqueous extract can be used in antimicrobial mouthwashes, prophylactic antibiotics, dental implants, and toothbrushing techniques after final approvals.

Data Availability

All data generated or analyzed during this study are included in this published article.

Consent

No consent was necessary.

Conflicts of Interest

All authors declare that they have no competing interests.

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