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RESEARCH PAPER

Investigating the Cytotoxic Effect of Tannic Acid Modified Silver Nanoparticles: in Vitro Assessment on MDCK Cell Lines

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ABSTRACT

Tannic acid plays a significant role in modifying silver nanoparticles due to its unique properties and functionalities. In this work, demonstrate the tannic acid modified silver nanoparticles exhibit different cytotoxicity compared to other silver nanoparticles due to the presence of the tannic acid coating. The tannic acid modification with silver nanoparticles can decrease their ability to inhibit the proliferation of MDCK cells line. Tannic acid itself has antioxidant properties and can scavenge free radicals, which may help mitigate the cytotoxic effects of silver nanoparticles. A green biosynthesis was used to prepare successful product and establish a tannic acid and silver nanoparticles (TA-AgNPs). TA-AgNPs was prepared in several concentrations (3.1, 6.25, 12.5, 25, 50) µg/mL and evaluated for cytotoxicity effect in vitro normal MDCK cell line. on the surface of silver nanoparticles tannic acid was effectively modified and characterized via UV Visible Spectroscopy, Fourier transform infrared (FTIR) spectroscopy, AFM, XRD, SEM and TEM. The observations with SEM indicated that produced TA-AgNPs had a spherical shape with a mean particle size of 13.55±5.9 nm. The cytotoxic effect and the anti-proliferative activity of TA-AgNPs treated MDCK cells showed the TA-AgNPs have less cytotoxicity and high bioavailability rate (90.66±0.7212) than AgNPs (90.65±0.8864) in the same concentration (50 µg/mL). This study revealed that tannic acid modified silver nanoparticles have a highly effective metal nanoparticles with promising new applications. Because of its significant unique properties, tannic acid may be referred to as the cross-linker in the future.

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INTRODUCTION

Nanotechnology is a rapidly developing field for new research that dealing with synthesis, design and modification of the particle structures with size approximately between (1-100 nm) [1].

Nanoparticles (NPs) are attracting much attention in many fields such as health care, food and feed, environmental health, chemical industries, space industries and drug-gene delivery due to their unique physical, chemical and biological properties[2,3].It would be ideal to synthesize of silver nanoparticles rapidly by employing a green chemistry method that also highly desirable, Silver nanoparticles (AgNPs) have been among the

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most engaging materials in medicine as a result of their cheap cost ,high efficiency, and uniquely optical and electronic properties, easy, cost-effective, economic, environmental friendly and sustainable technique, green biological synthesis was become one foremost interesting materials [4].Silver nanoparticles with the functionalization provided by tannic acid can be combined to produce type of nanomaterial referred to tannic acid modified silver nanoparticles. Tannic acid is a naturally occurring polyphenolic compound found in various plant sources, such as fruits, nuts, and tree barks [5].

The modification of silver nanoparticles with tannic acid involves coating the surface of silver nanoparticles with a layer of tannic acid molecules. This process is typically achieved by mixing a solution of silver nanoparticles and tannic acid that allowing them to react. The tannic acid molecules adsorb onto the surface of the silver nanoparticles, forming a stable coating [6].

The resulting tannic acid modified silver nanoparticles exhibit several unique properties and advantages. Some of these include:

Stability: The tannic acid coating enhances the stability of silver nanoparticles by preventing agglomeration or aggregation. This stability is particularly important for their applications in various fields [7].

Biocompatibility: Tannic acid is known for its biocompatibility, making the modified nanoparticles suitable for biomedical applications. The surface modification with tannic acid improves their compatibility with biological systems, reducing potential toxicity concerns [8].

Antimicrobial activity: Silver nanoparticles possess inherent antimicrobial properties [9]. The modification with tannic acid can enhance this activity, making tannic acid modified silver nanoparticles effective against a wide range of microorganisms such as bacteria, viruses, and fungi [10,11].

Surface functionality: Tannic acid contains multiple hydroxyl groups and aromatic rings, which provide functional groups for further modifications. These functional groups can be used to attach various molecules, such as drugs, targeting ligands, or biomolecules, expanding the wide variety applications of tannic acid modified silver nanoparticles [12].

Applications of tannic acid modified silver nanoparticles are diverse and include:

Biomedical applications: The antimicrobial properties and biocompatibility of tannic acid modified silver nanoparticles make them suitable for wound dressings, antibacterial coatings, and drug delivery systems [13,14].

Environmental applications: Tannic acid modified silver nanoparticles can be used in water treatment technologies to remove contaminants, such as heavy metals or organic pollutants, due to their adsorption and catalytic properties [15].

Food industry: Tannic acid modified silver nanoparticles have antimicrobial activity that can be utilized to extend the shelf life of food products by inhibiting the growth of spoilage-causing microorganisms [16].

Catalysis: Tannic acid modified silver nanoparticles have a functionalized that allow it to be employed for catalytic reactions, such as reduction or oxidation reactions, due to the presence of reactive functional groups [17].

Due to their easily to oxidation and agglomeration, which remarkably suppress their anti-bacterial and antiviral effects, AgNPs practical applications are currently restricted. The modification of AgNPs has attracted significant attention as a solution of these challenges. According to reports, that AgNPs modified with peptides, chitosan, polyethyleneimines and glucosamine have been shown enhanced anti-bacterial activity [18].

Right now, it's absolutely necessary to search for naturally compounds which are biocompatible and have antiviral and antibacterial properties that can protect human against pathogen. Natural compounds might be seen as having promise ones to support the fight against many diseases [19]. Tannic acid is a beneficial component can be provided in the valuable supplements and different types of useful materials, Tannic acid (TA), a polyphenol generated from plants, has antiviral activities and may be used as a cost-efficient adhesive for a variety of substrates [20].

Since tannic acid is one of the most common tannins that may be successfully produced from sources in nature with high efficiency, which is why scientists are interested in it. In addition, compared with Gallic acid, for example, it has large molecular weight. this leads to the research on it as an active compound of metal coatings and nanoparticles, or a biopolymer cross-linker [21].

Tannic acid has a lot of special properties. It has antitumor properties and antimutagenic. It

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also acts as an antioxidant, homeostatic agent and has activity against microorganisms (bacteria and viruses) [22].

Tannic acid modified silver nanoparticles generally exhibit reduced cytotoxicity compared to unmodified silver nanoparticles. Cytotoxicity refers to the potential of a substance to cause harm or toxicity to cells [23].

Unmodified silver nanoparticles can exhibit cytotoxic effects due to their direct interaction with cells and the release of silver ions, which can induce oxidative stress and damage cellular components [24]. However, the modification of silver nanoparticles with tannic acid can mitigate their cytotoxicity and enhance biocompatibility for several reasons:

Surface modification: The tannic acid coating on silver nanoparticles acts as a physical barrier, reducing direct contact between the nanoparticles and cells. This barrier can limit the interaction between silver nanoparticles and cellular components, thereby reducing cytotoxic effects [25,26].

Biocompatible properties of tannic acid: Tannic acid is known for its biocompatibility and low toxicity. By modifying silver nanoparticles with tannic acid, the overall cytotoxicity of the nanoparticles can be reduced. Tannic acid has been used in various biomedical applications and is generally considered safe for use [27].

Reduced silver ion release: The tannic acid coating on silver nanoparticles can also help reduced release of silver ions [28]. It is commonly recognized that a part of the cytotoxic effects of silver nanoparticles are caused by silver ions. The tannic acid coating can act as a barrier that hinders the release of silver ions and prevents their direct interaction with cells.

It's important to note that while tannic acid modification can reduce the cytotoxicity of silver nanoparticles, tannic acid modified silver nanoparticles specific cytotoxicity may still depend on factors such as the concentration of nanoparticles, exposure time, cell type, and the specific application context [29]. It is always necessary to conduct comprehensive cytotoxicity studies and evaluate the specific requirements of each application before using tannic acid modified silver nanoparticles in biological systems [30].

Moreover, tannic acid has the ability to reduce free radicals that caused different diseases development include Allergies, Cardiovascular, Diabetes [31]. It has also been proved that has anticancer properties. Tannic acid is also currently being investigated as an organic polymer addition because it has the ability to reveal bioactive properties and improve the advantages properties of materials used in biomedical applications. Thus, it is a really interesting active compound that can be utilized as a component in nutritional products and added to variety of consumables [32].

Tannic acid (TA) appears to be one of the most commonly and often utilized intermediate molecular mass polyphenols impacted in the generation of AgNPs. At the level of AgNPs synthesis, TA performs tow roles by reduced silver ions and formed stabilizes nanoparticles [33].

MDCK cells are easily available, frequently used in influenza studies and have previously received successful license for vaccine manufacturing. For MDCK cells that are suspended and fast-growing. More recently, the development of grow media led to MDCK culture cell lines that can proliferate capability as single cells up to concentrations which exceeds 10×10^6cells/ml [34].in the case of massive production, high maximum cells density and fast cells growth are essential to shorten the time needed for achieving the required producing scale[35].

A novel method that develops to produce silver nanoparticles without requiring any external energy or surfactant by utilized *E.coli* and silver salt solution (AgNO3). It has be found that adding bacteria broth to the metallic salt solution rapidly reduces it and modified AgNPs with tannic acid leading to the formation of highly stable metal nanoparticles. several characterization techniques were employed to analyze the final products of the synthesis which included UV-Visible spectroscopy, Fourier Transform Infrared spectroscopy (FTIR), X-Ray Diffraction (XRD), Atomic Force Microscope (AFM), scanning electron microscopy (SEM) and Transmission electron microscopy (TEM) to demonstration their morphology, chemical composition and cytotoxic activity in MDCK cell line.

Our purpose was to determine optimal concentration of TA-AgNPs with minimum cytotoxic impact in MDCK cell line to investigate there is a suitable therapeutic window.

MATERIALS AND METHODS

Reagents and instruments

Silver Nitrate ($AgNO_3$) was acquired from

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(Daejung Chemical Reagent Co. Int., Korea). Tannic acid ($C_{76}H_{52}O_{46}$), were purchased from (Aladdin Chemical Com.), Ethanol from (Duksan, Korea), Trypsin/EDTA(Capricorn, Germany), dimethyl sulfoxide (DMSO) was purchased from (Santacruz Biotechnology, USA) , RPMI 1640 (Capricorn, Germany) , 3-(4,5-dimethylthiazal-z-yl)-2,5-diphenyltetrazolium (MTT) stain and crystal violet were purchased from (Bio-World com.,USA), Fetal bovine serum were obtained from (Capricorn, Germany) Antibiotics include penicillin and streptomycin were procured from Biosource International (Nivelles, Belgium).

Escherichia coli was obtained from Al-Alawiya Children's Hospital, Bagdad, Iraq, for using in green synthesis of AgNPs. MDCK cells purchased from sigma – USA Co.

Ultraviolet spectrophotometry (UV-2450, Shimadzu, Japan), Dynamic light Scattering (Nano-ZS90, Malvern, England), Fourier Transform Infrared spectroscopy (FT-IR) (IRprestige-21, Shimadzu, Japan), X-ray diffraction (XRD) (Panalytical X'Pert PRO, UK), and atomic force microscope (AFM) (DualScopeTM DS) was used to characterize the synthesized TA-AgNPs. Ultra-high resolution field emission Scanning Electron Microscopy (SEM)(JSM-7800F, NEC Electronics Corporation, Japan) and transmission electron microscopy (TEM) (ZEISS LEO 912 AB, Germany) were used to investigated the morphologies of the TA-AgNPs. An inverted microscope (OLYMPUS IX71, Olympus, Japan) was used to visualize the shape of the MDCK cells after treated with AgNPs and TA-AgNPs.

Biosynthesis of Silver nanoparticles AgNPs by using E.coli

To prepare AgNPs[27], [36] by using *E.coli*, the large particles were separated from the bacterial broth using Centrifuge (Solution A). Separately, 3g of silver nitrate (AgNO3) was dissolved in 25 ml of deionized water using a magnetic stirrer for 30 minutes (Solution B). Then solution A was added to the precursor solution (B) drop by drop, and the solution immediately turned into a white gel. The gel was kept in the dark incubator at 37°C for 24 hours, and brown color precipitate was obtained. The precipitate was separated using Centrifuge and washed with deionized water and ethanol 6 times. The drying process was carried out using

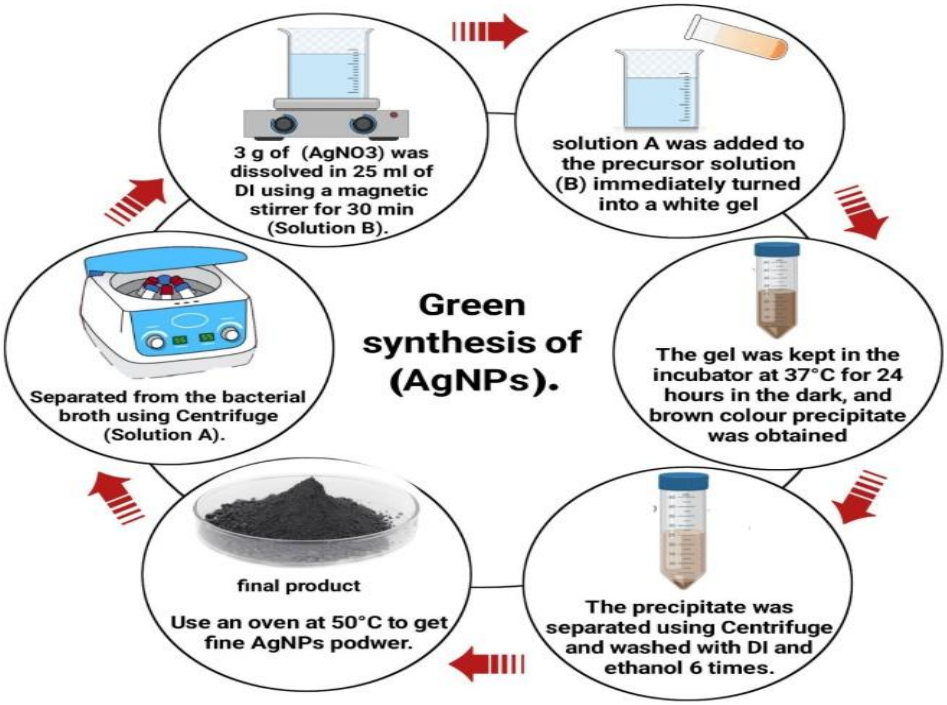

Fig. 1. Green synthesis of silver nanoparticles(AgNPs).

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an oven at 50°C to get fine Ag NPs powder**,** as described in Fig. 1 done by Adobe Photoshop CC 2019.

Preparation of Tannic acid-AgNPs

0.33 g of AgNPs was dispersed in 50 ml of deionized water using a magnetic stirrer for 45 min. Then 0.66 g of tannic acid was added to the AgNPs solution and stirred for 3 hours. After that, ultrasound was used for 30 minutes to obtain excellent dispersion. The solution is kept for 24 hours, after which the precipitate is separated using Centrifuge, then dried in the oven at 60°C, as shown in (Fig. 2) done by Adobe Photoshop CC 2019.

The synthesis of silver nanoparticles was carried out by reduction of AgNO3 with citrate and TA using a modification of the procedure show TA plays an important role in the formation of AgNPs with specific shape, size and size distribution [37]**.**

Characterization of AgNPs and Tannic acid modified AgNPs

Silver Nanoparticles was analyzed before and after modified with Tannic acid colloids via Ultraviolet-Visible spectrophotometer (UV-vis). Fourier infrared spectrometry (FTIR) used to record the surface functional groups of the TA-AgNPs. An X-ray diffractometer was employed to determine the crystalline state of the produced samples, an energy dispersive X-ray analysis (EDX) has been used to investigate the shape and metallic presence, performed. Via dynamic light scattering, the size distribution of the TA-AgNPs colloids have been determined. The morphology of the TA-AgNPs can be discovered by Ultra-high resolution field emission Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM).

Maintenance of cell cultures

Normal cell line MDCK cells were obtained from sigma–USA com. and were maintained in RPMI-1640 supplemented with 10% Fetal bovine serum, 100units/mL penicillin and 100µg/mL streptomycin· Cells were passaged using Trypsin-EDTA and that have been reseeded at 80% confluence twice a week, and incubated at 37°C [38,39].

Cytotoxicity Assays

The cytotoxic effect of (AgNPs and TA-AgNPs)

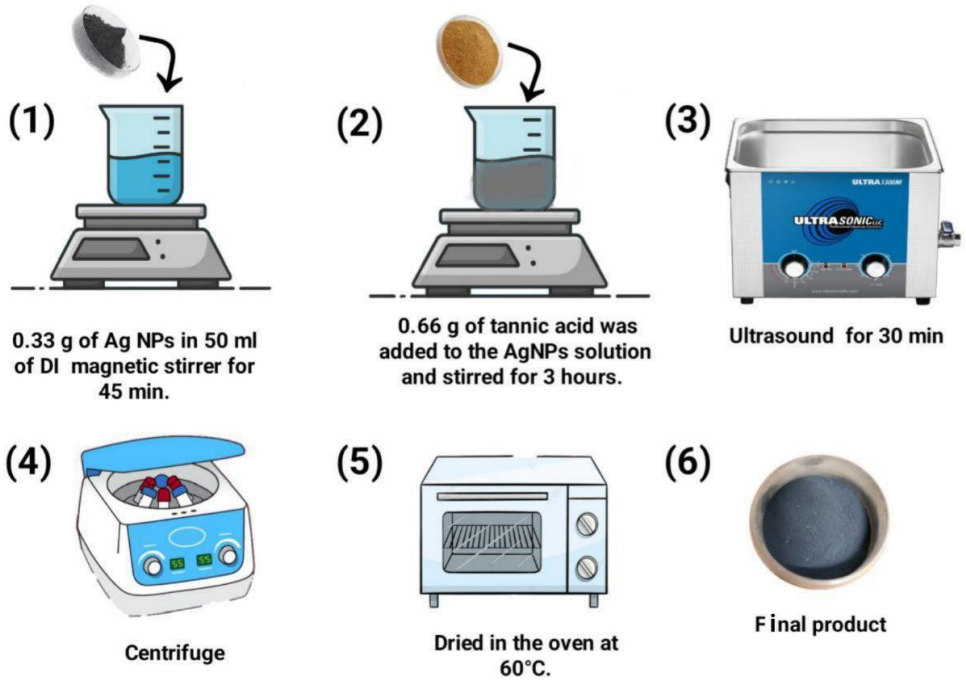

Fig. 2. Tannic acid modified AgNPs.

have been evaluated using the MTT assay in 96-well plates [40,41]. Cell line have been seeds at 1×10^4cells/well. After 24 hrs., AgNPs and TA-AgNPs was treated at different concentrations (3.1, 6.25, 12.5, 25, 50) µg/ml with cells until a confluent monolayer was formed. after 48 hours of treatment cell viability was measured by removing the medium, adding 100 µL of 2 mg/mL MTT solution then incubating the cells for 2.5 hours at 37°C. The crystals remaining in the wells after the MTT solution was removed were made soluble by adding 100 µL of DMSO (Dimethyl Sulphoxide) followed by 37°C for 15 min incubation with shaking [42]. The absorbency was measured at 492 nm using a microplate reader, the assay was carried out in triplicate.

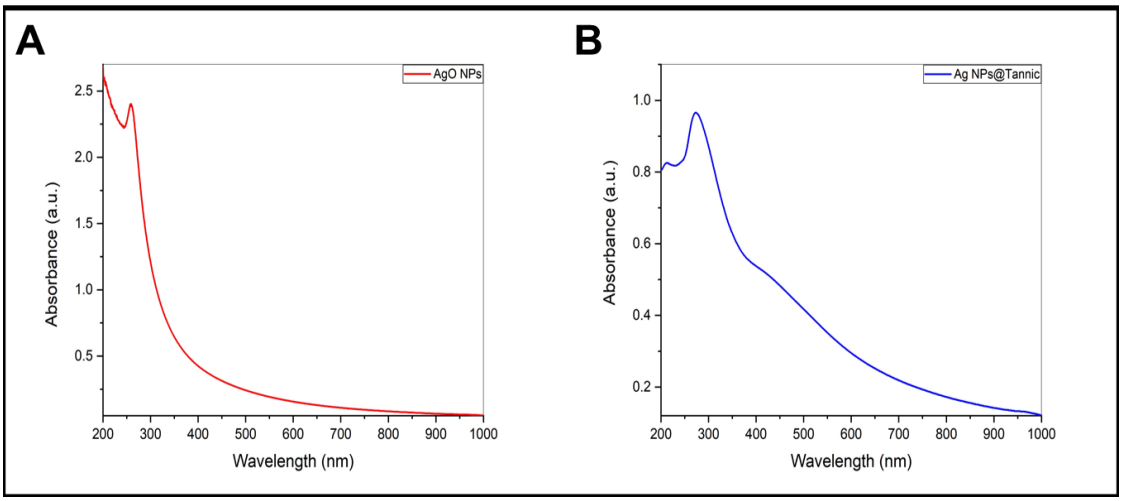

Fig. 3. UV–visible absorption spectra of (A) AgNPs and (B) TA-AgNPs.

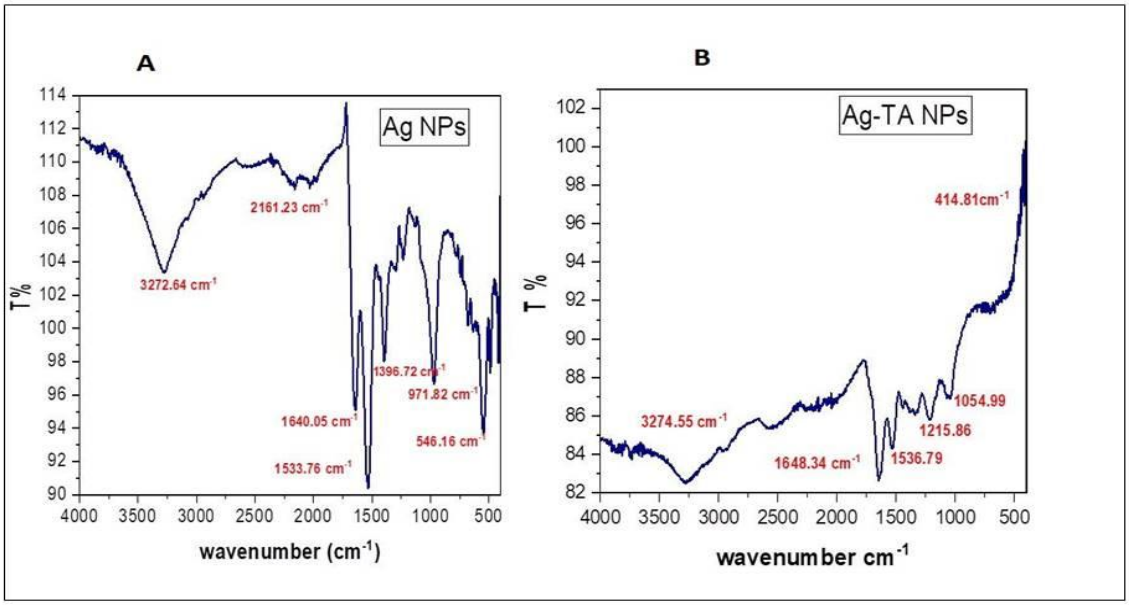

Fig. 4. FT-IR spectra (a) AgNPs and (b) TA-AgNPs.

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percentage of cytotoxicity (inhibition rate) of cell growth was calculated as the following equation [43,44]:

$$\text{Inhibition rate} = \text{A-B/A*100}$$

where A is the optical density of control and B is the optical density of the samples [45].

To visualize the shape of the cells under an inverted microscope, the cells were seeded into 24-well micro-titration plates at a density of 1×10^5 cells mL−1 and incubated for 24 h at 37 °C. Then, cells were exposed to AgNPs for 24hr. After the exposure time, the plates were stained with crystal violet stain and incubated at 37 °C for 10–15 min [43]. The stain was washed off gently using tap water until the dye was completely removed. An inverted microscope at 100× magnification was used to observed the cells, a digital camera that was attached to the microscope was used to captured the images [46-48].

Ethical approval

The study was conducted in accordance with the ethical principles and approved by Collage of Science for women, University of Babylon. The study protocol and the subject information were reviewed and approved by a local ethics committee according to the document number (16)/MSc research in (21/4/2024) to get this approval.

Statistical analysis

Statically the obtained data have been analyzed that use an unpaired t-test with GraphPad Prism 6[44]. The values were presented as the mean ± SD of triplicate measurements [49].

RESULTS AND DISCUSSION

Characterization of AgNPs and TA-AgNPs

The synthesis of TA-AgNPs was characterized in comparison with AgNPs using UV-Vis spectrum, FTIR, XRD, AFM, SEM, and TEM.

UV-Vis Spectrophotometry

The successful fabrication of AgNPs via tannic acid has been confirmed by UV-visible spectroscopy. As demonstrated with Fig. 3A, a maximum peak was observed at 275 nm, and spreading of the peak implied that the Ag NPs were polydispersed. On the other hand, Fig. 3B shows that using tannic acid as reducing and stabilizer agent caused spectral shift at 300 nm followed by broad peak ranging 400–450 nm relating to the Surface Plasmon Resonance (SPR band) of AgNPs.

The Surface Plasmon Resonance of Ag nanoparticles result in strong absorption in the UV/visible range. The UV-vis absorption spectrum of the resulting nanoparticles shows a broad peak

Table 1. Theoretical Parameters of AgNPs XRD.

Pos. [°2Th.]	(*hkl*)	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]	Crystalline Size (nm)
27.9	(110)	0.204672	3.20239	14.26	40.0
32.3	(111)	0.230256	2.77115	31.07	35.9
46.3	(211)	0.255840	1.96254	19.47	33.8
54.8	(220)	0.070299	1.67441	100.0	127.2
67.5	(222)	0.090000	1.38734	1.82	106.1

Table 2. Theoretical Parameters of TA-AgNPs XRD.

Pos. [°2Th.]	(*hkl*)	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]	Crystalline Size (nm)
27.9	(110)	0.179088	3.20502	55.76	45.7
32.3	(111)	0.179088	2.77500	100.00	46.1
46.2	(211)	0.127920	1.96433	58.08	67.5
57.5	(220)	0.358176	1.60263	15.33	29.1
67.5	(222)	0.307008	1.38863	6.61	31.1
74.5	(321)	0.818688	1.27344	4.12	12.2

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around 400 to 450 nm, which is attributable to surface plasmon resonance of silver nanoparticles. As the silver ions are reduced by tannic acid, silver nanoparticles are formed capped by tannic acid molecules. The capping provides stability and prevents aggregation. This result was in agreement with Srichaiyapol *et al*., which were reported that the maximum absorption peak for AgNPs synthesized with tannic acid was 240 nm [18]. Also, the largest peak absorption of 460 nm was recorded for AgNPs combined with tannic acid [6].

FT-IR Spectrum

The infra-red spectra of AgNPs and TA-AgNPs was investigated as shown in Fig. 4. Different peaks were recorded in AgNPs spectrum (Fig. 4A), which are: 3272.64 cm^{-1} represent NH stretching, 2161.23 cm^{-1} was assigned for OH vibration, 1640.05 and 1533.76 cm^{-1} represent the stretching of CC band, while 1396.72 cm^{-1} was assigned for CH vibration. The weak peaks of 971.82 to 419.00 cm^{-1} assigned to CH within plane bending vibrations. In Fig. 4B, the phenolic hydroxyl groups underwent oxidation, resulting in a weakening of the absorption bands at 3000–3500 cm^{-1} in an Infrared spectrum of TA-AgNPs. An absorption peak in 1648.34 cm^{-1} was detected, indicating the presence of the benzene ring skeleton stretching vibration peak. The shifts in peak positions and shapes indicate binding of tannic acid to Ag NPs. It confirms the presence of tannic acid and its interaction with Ag NPs during the synthesis [17].

X-ray Diffraction (XRD)

The crystalline nature of Ag NPs and TA-AgNPs was confirmed by X-ray crystallography. The XRD form of AgNPs (Fig. 5A) showed diffracted intensities documented at 2 theta angles (2θ) with four strong peaks: 27.9°, 32.3°, 46.3°, 54.8° and 67.5° resembles to the planes of (110), (111), (211), (220) and (222) respectively (Table 1), which correspond depending to face-centered cubic crystal structure of silver [50]. The average crystalline size of AgNPs calculated based on Debye-Scherrer formula was 68.6±44.55 nm.

The combination of tannic acid with Ag within nanoscale ranged resulted a crystalline diffraction pattern with 6 major peaks (Fig. 5B) including: 27.9°, 32.3°, 46.2°, 57.5°, 67.5° and 74.5° corresponds to the planes of (110), (111), (211), (220), (222) and (321), respectively (Table 2). The average crystalline size of Ag -TA NPs was 38.62±18.9 nm. Many researchers have noted that the face-centered cubic structure often exhibits the highest peak intensity for the (111) plane [51], [4, p. 201]. Consequently, it may be anticipated that the synthesized particles had a face-centered cubic structure for TA-AgNPs. Moreover, a slight background change in XRD pattern in TA-AgNPs

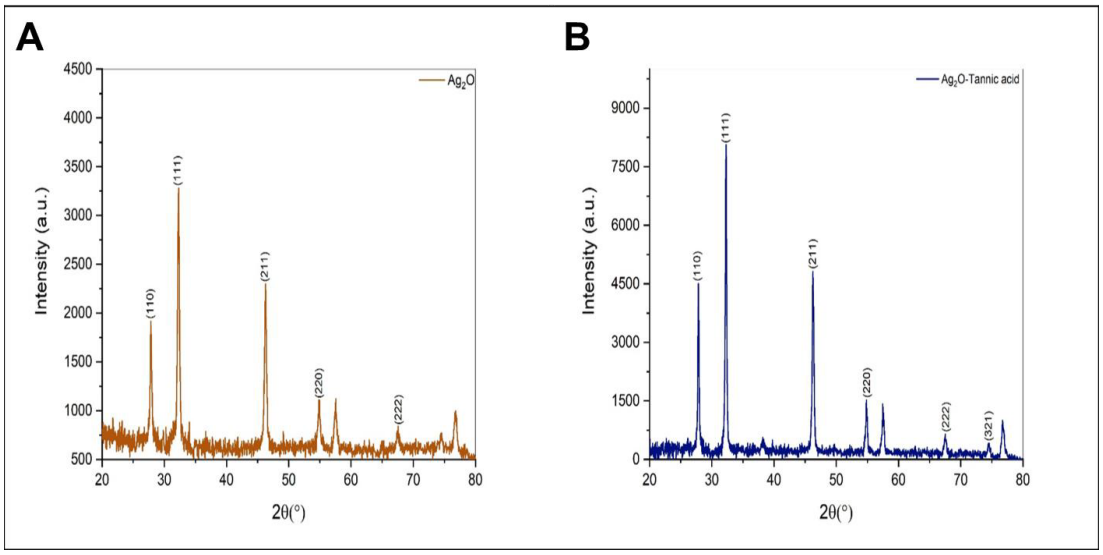

Fig. 5. (A) XRD diffraction pattern for AgNPs ,(B) : XRD diffraction pattern for TA-AgNPs.

compared to AgNPs may attributed to the formation of tannate, plane (321), which highly suggested the formation of nanocrystalline particles [52].

Atomic Force Microscopy (AFM)

The AFM technique is used to analyze the surface morphology and average size of nanoparticles in the samples. The AgNPs that were prepared were analyzed using AFM. The image shown in Fig. 6A displays the morphology of the AgNPs, while Fig. 6B represents the estimated average diameter of the AgNPs. The measured average diameter, determined using AFM software, is 156.3 nm. It seems that the AgNPs exhibit a uniform distribution with medium clusters and aggregates.

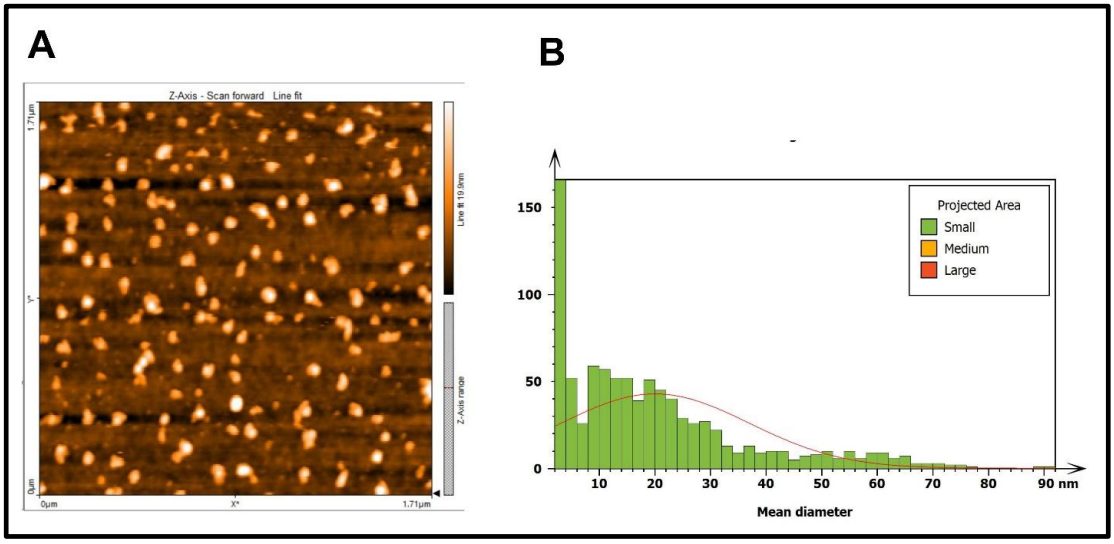

Fig. 6. AFM topographical analysis of AgNPs (A) 2D image (B) Distribution chart of particle size in nm.

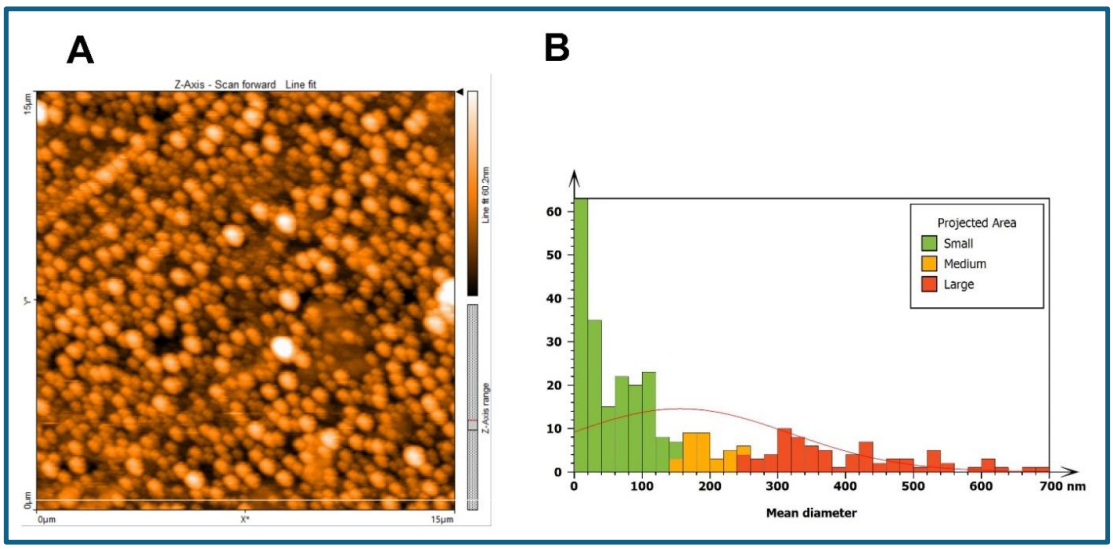

Fig.7. AFM topographical analysis of TA-AgNPs (A) 2D image (B) Distribution chart of particle size in nm.

The results indicate that AgNPs have a spherical topology and morphology.

AFM results are in agreement with different AFM reports utilizing Ag NPs in AFM analysis, which showed a spherical topology with average size of 46.5 nm [53].

On the other hand, AFM results of TA-AgNPs in Fig. 7A, and B, showed that TA-AgNPs have semi-spherical shape with smooth topography and no aggregates. The average size of TA-AgNPs was 20.06 nm.

AFM provides high resolution 3D topographic images of the nanoparticles deposited on a flat substrate. The particle height and radius can be measured from cross-sectional analysis. AFM images show individual nanoparticles as well as their aggregates. The shape can be visualized and determined to be spherical or irregular. Particle size distribution is obtained by measuring the dimensions of a large number of particles. AFM provides quantitative topographical data on the nanoparticles and confirms their nanometer scale

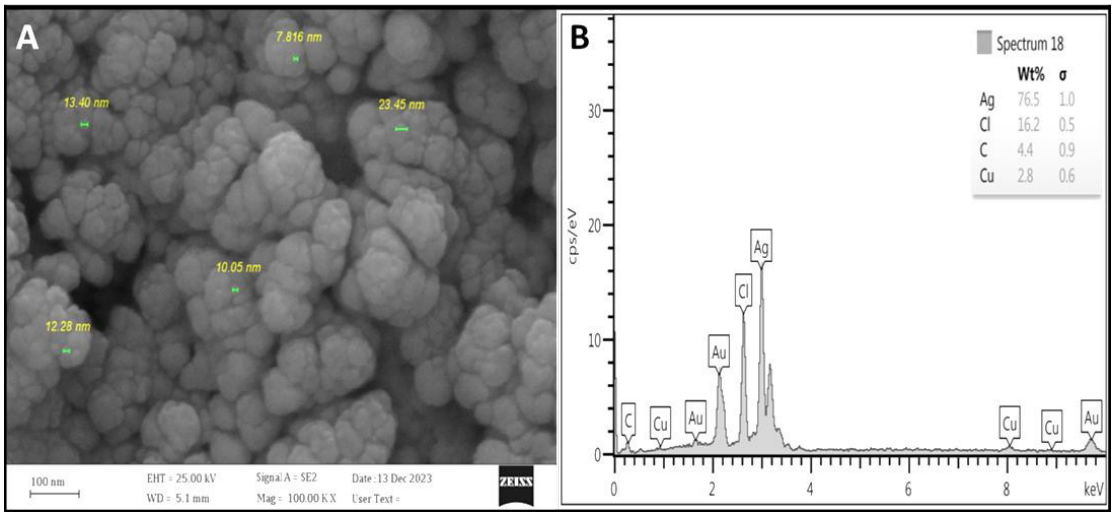

Fig. 8. SEM image of AgNPs , EDX spectrum for AgNPs showing the presence of Ag and other elements.

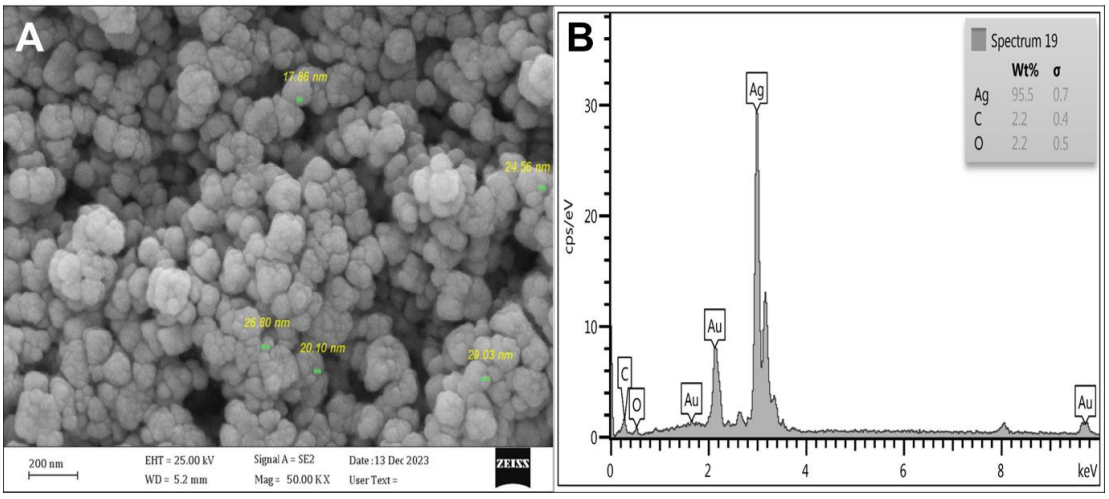

Fig. 9. (A) SEM image of TA-AgNPs , (B) EDX spectrum for TA-AgNPs showing the presence of Ag and other elements.

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size [54].

Tannic acid acts as both a reducing and capping agent in the green synthesis of silver nanoparticles [55]. The gallic acid residues in tannic acid reduce silver ions to silver atoms which nucleate to form nanoparticles. The abundant phenolic hydroxyl groups of tannic acid also bind to the nanoparticle surface, providing steric stabilization. This

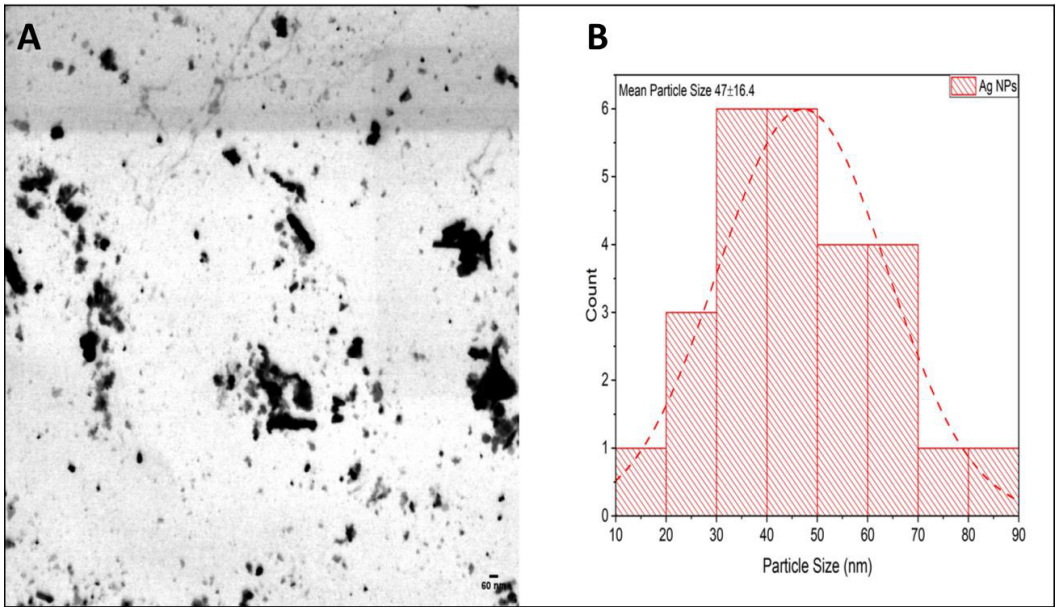

Fig. 10. TEM images and particle size distribution of (A) AgNPs and (B) AgNPs histogram.

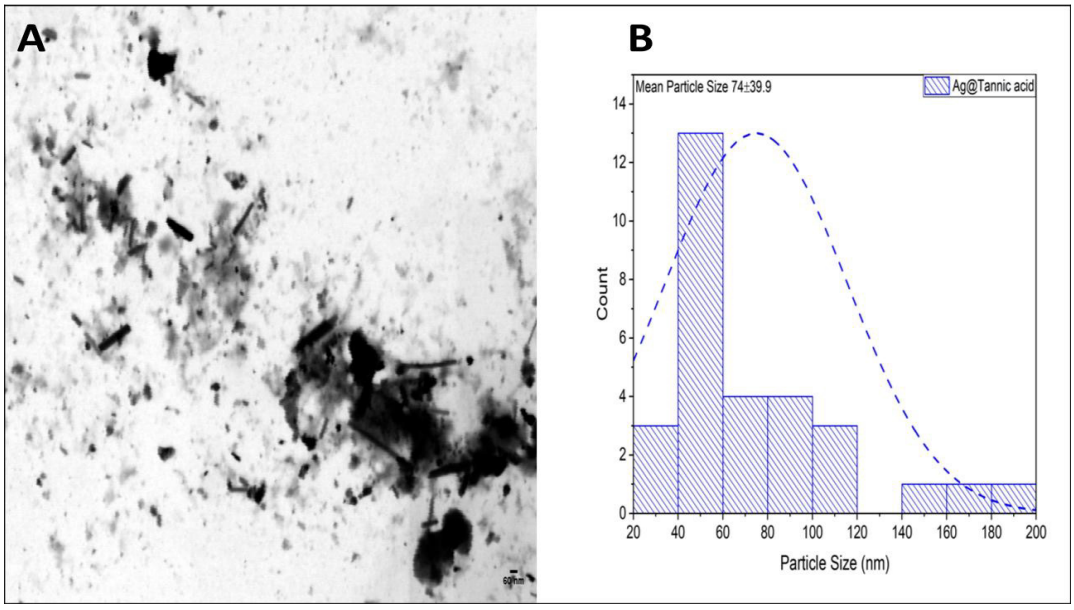

Fig. 11. TEM image and particle size distribution of TA-AgNPs , (B) TA-AgNPs histogram. Figure 12: Cytotoxicity of AgNPs in MDCK cells.

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prevents uncontrolled growth and aggregation of the nanoparticles. The capping effect of tannic acid limits the particle size to the nanometer scale, which highly confirmed by AFM results. Using tannic acid instead of other reducing agents like sodium borohydride results in smaller AgNPs with a narrower size distribution [21].

Scanning Electron Microscope (SEM)

Images of SEM were used to determine the surface morphology, size and shape of (AgNPs and TA-AgNPs). Fig. 8A shows the presence of smooth structure with high aggregation and spherical shaped AgNPs with an average size of 13.55±5.9 nm. The agglomeration of AgNPs was previously reported, and observations indicate that the majority of particles have a spherical form with size distribution of the particles ranging from 10 to 25 nm [56]. Rautela *et al*., [57] also reported that SEM images of AgNPs showed spherical particles morphology with particle size below 100 nm [57]. Elemental analysis of AgNPs was detected using energy dispersive X-ray analysis (EDX) equipped with SEM. Results in Fig. 8B shows the presence of 76.5% Ag followed by Cl (16.2%), C (4.4%) and Cu (2.8%). The elemental composition of AgO NPs verified an intense peak at 3 KeV, which is characteristic of the absorption of metallic AgNPs. The presence of other elements can be attributed to impurities resulting from pervious analysis.

TA-AgNPs of spherical nanoscale particles have been noticed with an average size of 24.66±4.6 nm (Fig. 9A, and B), which caused size shifting as a result of conjugation with the tannic acid. However, TA-AgNPs showed more mono-dispersity when compared with the AgNPs.

The elemental analysis of TA-AgNPs showed that 95.5% of the detected spectrum was Ag. A sharp peak at 3 KeV confirmed the existence and purity of AgNPs.

F-Transmission Electron Microscope (TEM)

The size and shape of AgNPs and TA-AgNPs was further detected using transmission electron microscope (TEM). Results in Fig. 10 exhibit that AgNPs mainly consist of ting nanocluster with

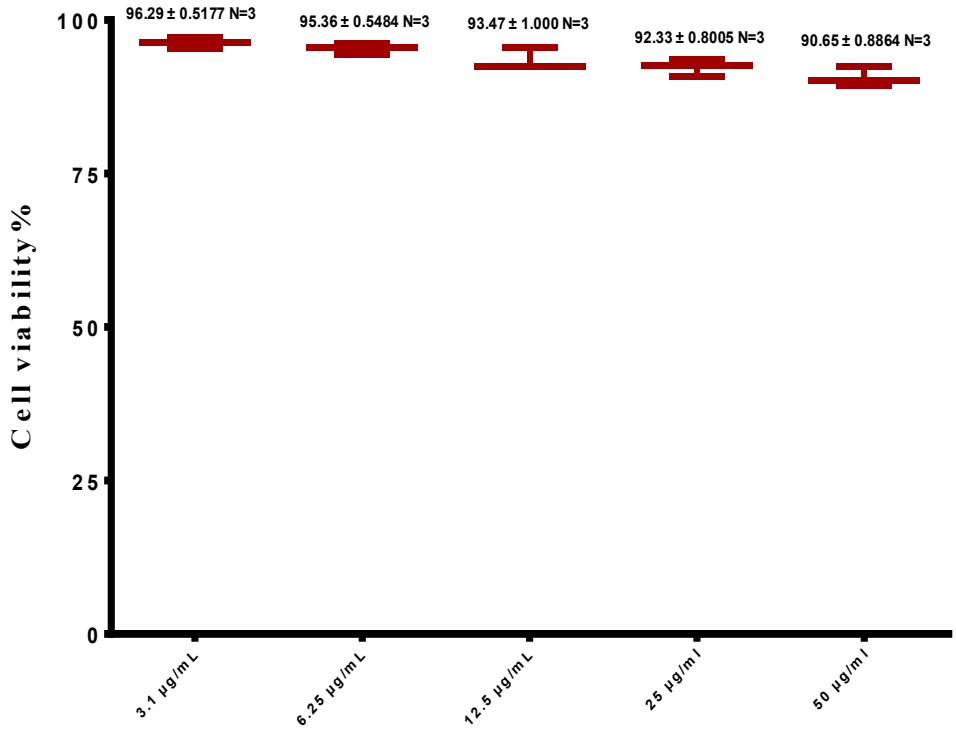

Fig.12. Cytotoxicity of TA-AgNPs in MDCK cells.

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average size of 47±16.4 nm. The nanoclusters have agglomerated, resulting in the formation of bigger particles. This phenomenon was previously identified by Goudarzi *et al*., which they indicated that TEM images of AgNPs showed large aggregations of particles that stuck together into bigger nanoparticles [58].

In Fig. 11, the TEM image of TA-AgNPs showed that the TA-AgNPs particles formed clusters as a result of the close proximity of nanoparticles with an average size of 74±39.9 nm. Such observation was suggested that such clustering hindered the accurate measurement of particle size, which is consistent with the broad band behavior seen for TA-AgNPs[26]. Furthermore, the drying procedure used in TEM sample preparation might lead to non-uniform deposition, causing nanoparticles to agglomerate as the solvent evaporates [59].

Cytotoxicity result

The cytotoxic effect of Ag NPs and TA-AgNPs against MDCK cells was studied. The anti-proliferative activity and ability of the AgNPs and TA-AgNPs to inhibition of MDCK cell line proliferation was tested. The study results were shown in Figs.12-14.

Investigation cytotoxicity of silver nanoparticles at the concentrations (3.1, 6.25, 12.5, 25, 50 µg/mL) on MDCK cells revealed different toxicity rates, that it was noticed toxicity percent as follows: 3.71% at concentration 3.1 µg/mL, 4.64% at concentration 6.25 µg/mL, 6.53% at concentration 12.5 µg/mL ,7.67% at concentration 25 µg/mL and 9.35% at the highest concentration 50 µg/mL as shown in (Fig. 12).

In Fig. 13 detected cytotoxicity of silver nanoparticles modified with tannic acid (3.1, 6.25, 12.5, 25, 50 µg/mL) on MDCK cells shown different toxicity values, that it was noticed toxicity percent as follows: 5.29 % at concentration 3.1 µg/mL, 7.05% at concentration 6.25 µg/mL, 8.33% at concentration 12.5 µg/mL ,9,02% at concentration 25 µg/mL and 9.34% at the highest concentration 50 µg/mL.

When comparing the results in the tow Figs. 12, and 13 can be conclude the higher concentration of TA-AgNPs 50 µg/mL is less cytotoxicity from 50 µg/mL AgNPs. In other previous studies were

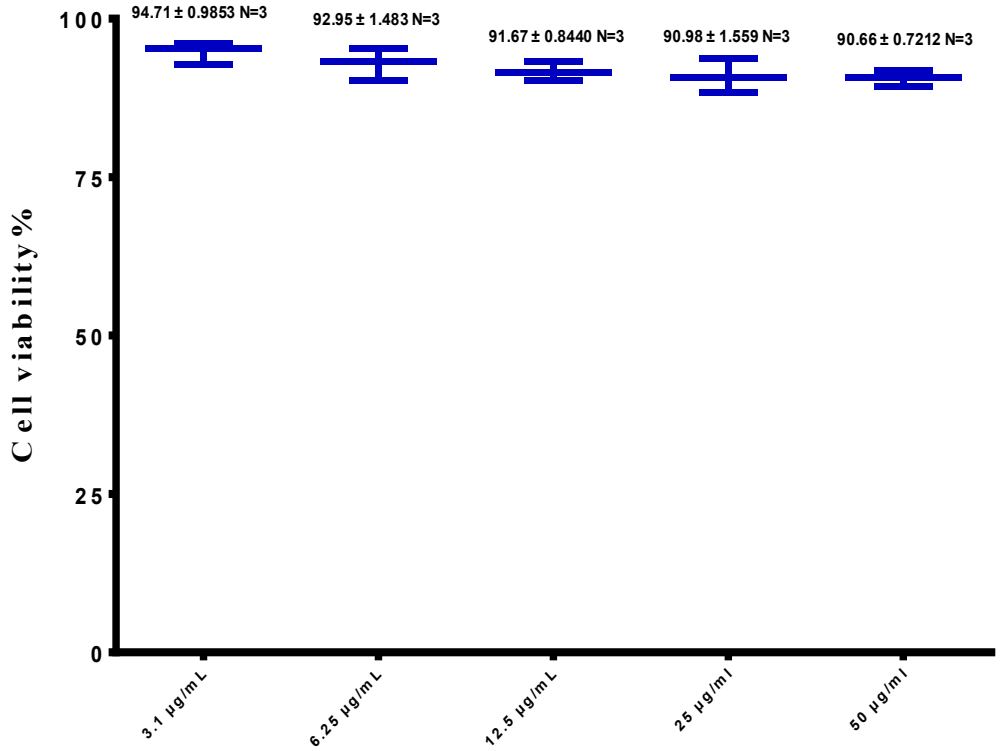

Fig. 13. Cytotoxicity of TA-AgNPs in MDCK cells.

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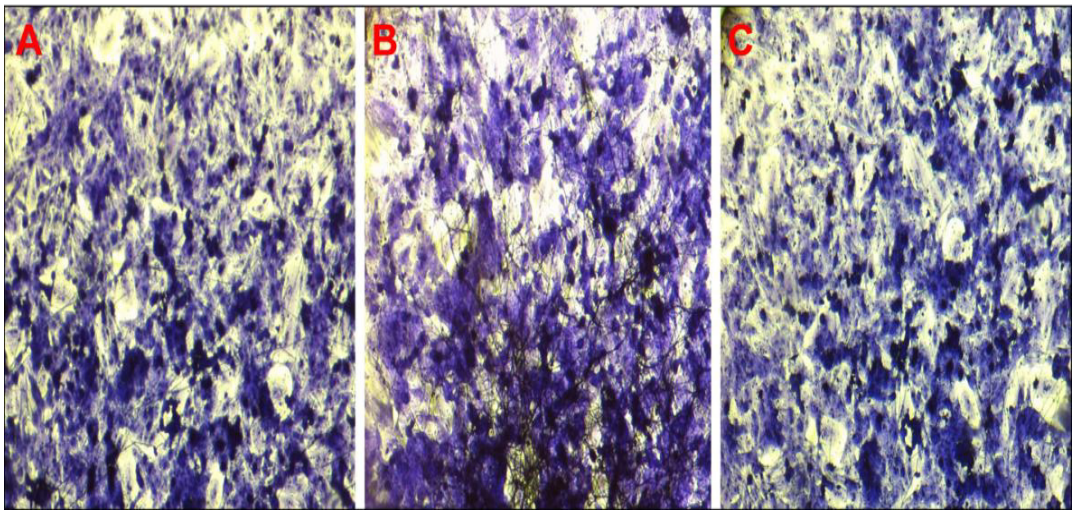

Fig. 14. (A) Control Un-treated MDCK cells , (B) Morphological changes in MDCK cells after treated with AgNPs , (C) Morphological changes in MDCK cells after treated with TA-AgNPs.

confirmed that TA-AgNPs showed less cytotoxicity to human cells in comparison with pure AgNPs [53], [60].

Morphological observation of treated MDCK cells with AgNPs clearly showed moderate cytoplasmic condensation and cell shrinkage (Fig. 14B) when compared with control un treated MDCK cells morphology (Fig. 14A), while (Fig. 14C) show a few morphological changes in MDCK cell treated with TA-AgNPs. These results further agree with previous findings, where it was indicated that the AgNPs was not toxic to MDCK cells [61].

However, there have been studies in the scientific literature that have investigated the cytotoxicity of tannic acid modified silver nanoparticles compared to other types of silver nanoparticles. The results of these studies have indicated that tannic acid modification can indeed reduce cytotoxicity.

For example, Poojari *et al*., (2016) evaluated the cytotoxicity of tannic acid modified silver nanoparticles and compared them to unmodified silver nanoparticles. The results showed that tannic acid modified silver nanoparticles exhibited significantly lower cytotoxicity compared to unmodified silver nanoparticles in various cell lines [8].

Another investigated the cytotoxicity of tannic acid modified silver nanoparticles and compared them to citrate-capped silver nanoparticles. The findings demonstrated that tannic acid modified silver nanoparticles had reduced cytotoxicity and improved biocompatibility compared to citrate-capped silver nanoparticles [17].

These studies provide evidence supporting the reduced cytotoxicity of tannic acid modified silver nanoparticles compared to other types of silver nanoparticles. However, it's important to note that the specific cytotoxicity profile can vary depending on various factors, including the experimental conditions, nanoparticle concentration, cell type, and assay methods used [62].

Here are a few examples of studies related to the cytotoxicity of tannic acid modified silver nanoparticles:

Gherasim *et al*.,2020, study compared the cytotoxicity of tannic acid modified silver nanoparticles with bare silver nanoparticles in human lung epithelial cells. The researchers evaluated cell viability, ROS generation, and oxidative stress markers [63].

The study of Shi *et al*. (2018) and Kis *et al*. (2022) compared the cytotoxicity of tannic acid modified silver nanoparticles with other surface-coated silver nanoparticles in human breast cancer cells. The researchers assessed cell viability, apoptosis induction, and cellular uptake [64,65].

CONCLUSION

TA-AgNPs were produced by a novel green synthesis of AgNPs and modification with tannic acid, resulting in spherical and crystalline

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nanoparticles with an average size of 24.66±4.6 nm. This allowed TA-AgNPs to be used in vitro safely on the MDCK cell line at the cytotoxic concentration CC50 (50 µg/mL). TA-AgNPs are highly stable and prevent aggregation of AgNPs for a long time, providing a significant benefit for biological synthesis Additionally, the high percentage survival rate of 90.66±94.71% for biogenic TA-AgNPs suggests that neither AgNPs nor TA-AgNPs have any effect on the viability of MDCK cells, which proliferate extremely efficiently. So we now report the green synthesis of silver nanoparticles for the first time, which do not show any cytotoxicity on MDCK cells. These findings suggest that TA-AgNPs may not be hazardous to normal human cells.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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