Int. J. Nano Dimens., 11 (2): 158-167, Spring 2020

ORIGINAL ARTICLE

Biosynthesis of Silver nanoparticles and investigation of genotoxic effects and antimicrobial activity

Hayrunnisa Nadaroglu[1, 2*], Azize Alayli[2, 3], Selcuk Ceker[4], Hatice Ogutcu[5], Guleray Agar[6]

[1]Department of Food Technology, Erzurum Vocational Collage, Ataturk University, 25240, Erzurum, Turkey

[2]Department of Nano-Science and Nano-Engineering, Institute of Science and Technology, Ataturk University, 25240 Erzurum, Turkey

[3]Department of Nursing, Faculty of Health Sciences, Sakarya University of Applied Sciences, 54187, Sakarya, Turkey

[4]Department of Pharmaceutical Basic Sciences, Faculty of Pharmacy, Agri İbrahim Cecen University, 04000, Agrı, Turkey

[5]Ahi Evran University, Faculty of Agriculture, Department of Field Corps, 40200 Kırsehir, Turkey

[6] Department of Biology, Faculty of Science, Ataturk University, 25240 Erzurum, Turkey

Received 09 December 2019; revised 22 April 2020; accepted 23 April 2020; available online 24 April 2020

Abstract

Health risk assessment of nanomaterials is a new and important area emerging; obtaining nanoparticles by green synthesis method and performing cytotoxicity, genotoxicity and antimicrobial testing is an important endpoint. In vitro studies for nanoparticles (NPs) obtained by the non-toxic method offer many advantages, such as the study of the bioavailability of nanomaterials to sensitive target cells. It will be useful for investigating the toxic and genotoxic risks associated with nanoparticle exposure. In this study; silver nanoparticles (AgNPs) were synthesized by green synthesis using grape vinegar prepared by ourselves. The resulting Ag NPs were characterized using Fourier transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM) and X-ray powder diffraction (XRD) methods and for different AgNPs concentrations in the range of 5-60 nm. The genotoxic effects of AgNPs were investigated using the Sister chromatid exchange (SCE) test and Micronucleus (MN) tests. Furthermore, the antibacterial and antifungal activities of the synthesized compound were tested against some pathogenic bacteria which are causative agents of the disease. As a result; it was found that the synthesized compound showed different degrees of inhibitory effect on the growth of pathogen strains compared to standard antibiotics. The findings are thought to provide clinically useful information in the treatment of many diseases using AgNPs at optimum concentrations (non-genotoxic concentrations).

Keywords: *Antimicrobial Activity; Green Synthesis; Pathogenic Microorganism; Silver Nanoparticles; Vinegar; Genotoxic Effect.*

How to cite this article

Nadaroglu H, Alayli A, Ceker S, Ogutcu H, Agar G. Biosynthesis of Silver nanoparticles and investigation of genotoxic effects and antimicrobial activity. Int. J. Nano Dimens., 2020; 11 (2): 158-167.

INTRODUCTION

Advances in nanotechnology provide improvements in a number of properties (magnetic, catalytic, optical, electrical and mechanical) of materials of the same formulation. Metal NPs have a very high surface area and surface-atomic fraction. By decreasing the size of NPs, they acquire physicochemical properties, reactive effects, visual properties, electronic properties, and anti-bacterial properties [1, 2].

NPs are synthesized by making use of different methodologies including chemical, physical and biological [3]. Synthesis of high amounts of NPs using chemical methodology can be carried out in a short time. However, chemicals used for reduction

* Corresponding Author Email: *hnisa25@atauni.edu.tr*

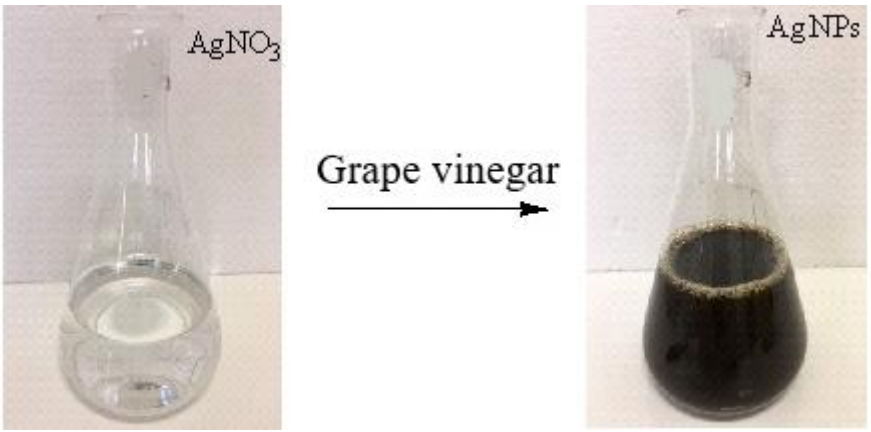

Fig. 1. Green synthesis of AgNPs.

are lethal and lead to non-environmentally friendly results. These drawbacks have led to increased interest in the use of ecological non-toxic methods in the synthesis of NPs [4–7].

Therefore, in recent years, there is increasing interest in the use of environmentally friendly, economical and non-toxic "green synthesis method" in the synthesis of NPs [8]. For this purpose, NPs were synthesized *in vitro* and *in vivo* using many plants, fungi and bacteria [9, 10].

AgNPs from metal NPs have limited effect on numerous bacterial progeny and microorganisms that occur in biological and industrial procedures due to their high antimicrobial properties [11]. AgNPs are used in medical equipment and cream and skin balm applied to open wounds to prevent bacterial contamination [12, 13].

In addition, in the textile industry, where AgNPs are used the most, antibacterial products (sports equipment, swimsuits, bandages, etc.) are produced [14]. Although AgNPs have many benefits, their side effects are not fully known and the risk of reaching the cell nucleus and damaging the genetic material [15].

However, there are some studies suggesting the widespread use of Ag nanoparticles, hepatotoxicity, neurotoxicity, nephrotoxicity and genotoxicity [16]. The genotoxicity and cytotoxicity of Ag nanoparticles have been demonstrated in both in vitro and in vivo assay systems. Induction of chromosomal and DNA damage by Ag NPs has been studied in many mammalian and fish cells [17].

In this study; AgNPs were synthesized by green synthesis using grape vinegar. The resulting AgNPs were characterized structurally and morphologically (using Fourier transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM) and X-ray powder diffraction (XRD) methods. In the last stage of the research; Sister chromatid exchange (SCE) and Micronucleus (MN) assays were performed to investigate the genotoxic effects of AgNPs for different AgNPs concentrations in the range of 5-160 mg/mL. Synthesized AgNPs were investigated for antimicrobial activity against pathogenic strains (Gram (-) and Gram(+) bacteria and yeast); *Staphylococcus aureus, Staphylococcus epidermis, Pseudomonas aeroginosa, Escherichia coli*, *Salmonella typhi H, Micrococcus luteus, Bacillus cereus, Klebsiella pneumonia, Proteus vulgaris*, *Brucella abortus* and *Candida albicans*.

MATERIALS AND METHODS

Synthesis of silver nanoparticles

AgNPs were performed using the procedure in the literature of Nadaroglu *et al.*, [6]. Briefly; 20 mL of grape vinegar was added to 80 mL of 0.1 M $AgNO_3$ solution and the mixture was stirred at room temperature with a magnetic stirrer. The color of the solution changed from colorless to light yellow and then to brown. The formation of silver nanoparticles was followed by color change. Separation of the silver nanoparticles from the dispersion was carried out by centrifugation, after which the AgNPs were washed several times with distilled water and then with acetone to remove water insoluble impurities. The resulting Ag NPs were lyophilized for analysis and stored in dry vials (Fig. 1).

Synthesis and characterization of AgNPs

For identification of AgNPs in solution, the wavelength (λ) was determined using the Epoch Nanodrop UV-Vis spectrometer in the 300-900 nm range. Characterization of surface chemistry of the AgNPs and biomolecules in the grape vinegar solution was performed using a Fourier transform infrared spectroscopy (Bruker VERTEX 70v). FT-IR spectra were recorded at 4000-400 cm^{-1}. The shape, size and surface of the synthesized AgNPs were analyzed using scanning electron microscopy (SEM) with high resolution images using Zeiss Sigma 300 scanning electron microscope (SEM), and the selected area was X-ray diffraction (XRD) analysis to determine synthesized phase distribution, crystallinity and purity using PANanlytical Empyrean X-Ray Diffractometer (UK). It was used to calculate the average silver nanoparticle size with the help of the Debye-Scherer equation.

In vitro micronucleus and sister chromatid exchange tests

The method developed by Fenech [18] was modified to determine the number of micronucleus. To the prepared solution, the agents to be tested was added and cultured at 37 °C for 72 h in a 5% CO_2 moist atmosphere [19]. Then, cytokalazine-B was added 28 h. before harvesting to all culture tubes with a final concentration of 3 µg/mL. After 72 h. incubation, the cells were harvested by centrifugation (1000 rpm, 10 min.) and the supernatant was removed. 6 mL of hypotonic solution (0.05 M KCl) was added to the pellet containing lymphocyte cells, vortexed and incubated at 37 °C for 7 min. After the incubation period, the lymphocyte cells were harvested by centrifugation and the supernatant was removed. 6 mL of fresh fixative solution was added drop by drop to the pellet. The fixation procedure was repeated three times and the tube was centrifuged. The cell pellet was re-suspended in 1 mL of fresh fixative solution, and then the suspension was dropped on to clean and labeled microscope slides and incubated at room temperature for 72 h. After the incubation period, the slides were stained with 5% giemsa dye solution for 10 min and excess giemsa dye was removed with distilled water. The slides were air-dried and only bi-nucleated cells were scored for MN analysis. For each experimental group, approximately 1000 bi-nucleated cells were analyzed for the presence of MN [20].

For SCE, 5-bromo 2-deoxyuridine (BrdU) at 8 mg/mL was added at the initiation of cultures. All cultures were kept in dark to avoid damage to the BrdU and then, 0.1 mg/mL of colcemid was added 3 h. before harvesting to arrest the cells at metaphase. The cultures were centrifuged at 1000 rpm for 10 min. Cells were harvested and treated for 25 min. with hypotonic solution and fixed in a 1 : 3 mixture of acetic acid/methanol (v/v). BrdU incorporated metaphase chromosomes were stained with the fluorescence plus Giemsa technique as described by Perry and Evans [21]. For each cell culture, 30 metaphase with at least 43 chromosomes with good distribution were evaluated in light microscope [22]. The color continuity of staining in the chromatids was impaired, and each point where there was a change of color between the chromatids was evaluated as a fracture [23].

Antimicrobial activity

Test microorganisms

The pathogenic bacteria cultures; *Staphylococcus aureus* ATCC25923, *Staphylococcus epidermis* ATCC12228, *Escherichia coli* ATCC1280, *Salmonella typhi* H NCTC901.8394, *Micrococcus luteus* ATCC9341, *Bacillus cereus* RSKK-863, *Klebsiella pneumonia* ATCC 27853, *Proteus vulgaris* RSKK 96026, *Pseudomonas aeroginosa* sp. *Brucella abortus* RSKK03026 and *Candida albicans* Y-1200-NIH yeast were used.

Detection of antimicrobial activity

The synthesized compound was examined for their antimicrobial activity by the well-diffusion method against gram-negative bacteria (*S. typhi H, E. coli*, *K. pneumonia*, *P. vulgaris*, *P. aeruginosa and B. abortus),* gram-positive bacteria (*S. aureus, S. epidermis, M. luteus, B. cereus*) and one yeast (*C. albicans*) [24–28].

The compound was kept dry at room temperature and dissolved (0.25 µg/mL) in DMSO. DMSO was used as solvent for compounds and also for control. DMSO was found to have no antimicrobial activity against any of the tested organisms. 1% (v/v) of 24 h. broth culture (pathogenic bacteria and yeast) containing 10^6 CFU/mL was placed in sterile plates. Mueller-Hinton Agar (MHA) (15 mL) kept at 45 °C was then poured into the petri dishes and allowed to solidity. Then wells of 6 mm diameter were punched carefully by using a sterile cork borer

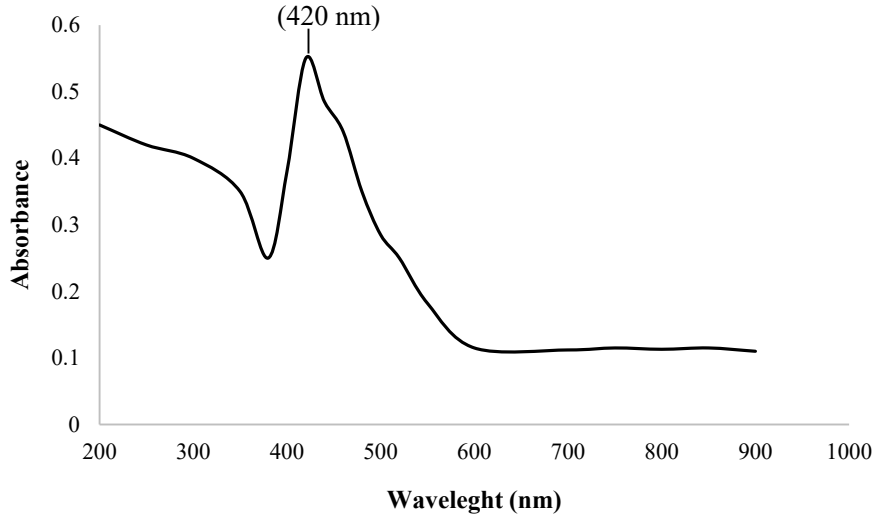

Fig. 2. UV spectrum of silver nanoparticles.

and were entirely filled with the synthesized compounds. In last stage, plates were incubated for 24 h at 37 °C on the incubator. At the end of the incubation period, the mean value obtained for the two well was used to calculate the zone of growth inhibition of each sample.

The pathogenic bacteria cultures and yeast were tested for resistance to five standard antibiotics produced by Oxoid Lt., Basingstoke, UK. These were: Ampicillin, Nystatin, Kanamycin, Sulphamethoxazol and Amoxycillin [29–34].

RESULTS AND DISCUSSION

Synthesis of silver nanoparticles

The formation of silver nanoparticles in the medium was determined mainly by color change. The color changes in the reaction medium function as the reducing power of silver ions (Ag^+) of some chemical compounds such as alkaloids, flavonoids, saponins, steroids and color found in grape vinegar. Visually; when the grape vinegar was treated with 0.1 M silver nitrate aqueous solution, colorless to light yellow coloration was observed within the first few minutes, then the solution turned from light yellow to brown. It then turned dark brown when the reaction was continued all night at 25 ° C (Fig. 1). Silver nanoparticles are due to the surface plasmon resonance in this brown, aqueous solution [35-36].

Characterization

UV-Visible absorption spectrum

Optical properties of silver nanoparticles were investigated by absorption spectroscopy. For this purpose, wavelength scanning for Ag NP formed in the reaction medium Surface Plasmon resonance bands plays a vital role in terms of size, shape and morphology. Fig. 2 shows the UV-visible spectra of nanoparticles obtained by cimin grape vinegar. The synthesized Ag nanoparticles gave a strong plasmon resonance band at 424 nm. UV-Vis spectroscopy has generally been used to detect the presence of AgNPs by green syntheses. In particular, the absorbance between 400 nm and 450 nm was generally used as an indicator to prove the reduction of Ag^+ of metallic Ag [35].

Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy measurements were performed to determine possible biomolecules responsible for the reduction and effective stabilization of silver nanoparticles [35]. In green synthesis, there are many functional groups that may be responsible for the bi-reduction of Ag^+ ions. Bands in different regions of the spectrum for grape vinegar and silver NPs were analyzed and shown in Fig. 3.

The FTIR spectrum of the extract of grape shows the vibrations of different main bands at

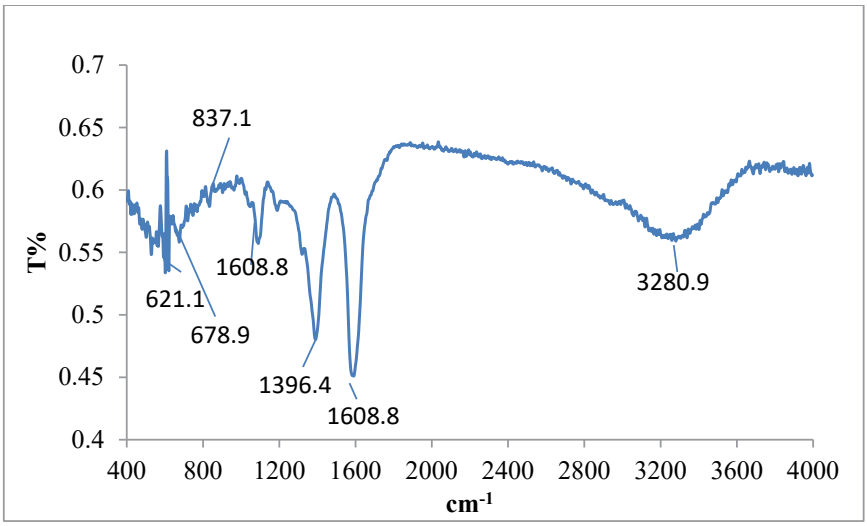

Fig. 3. FTIR spectra of silver nanoparticles.

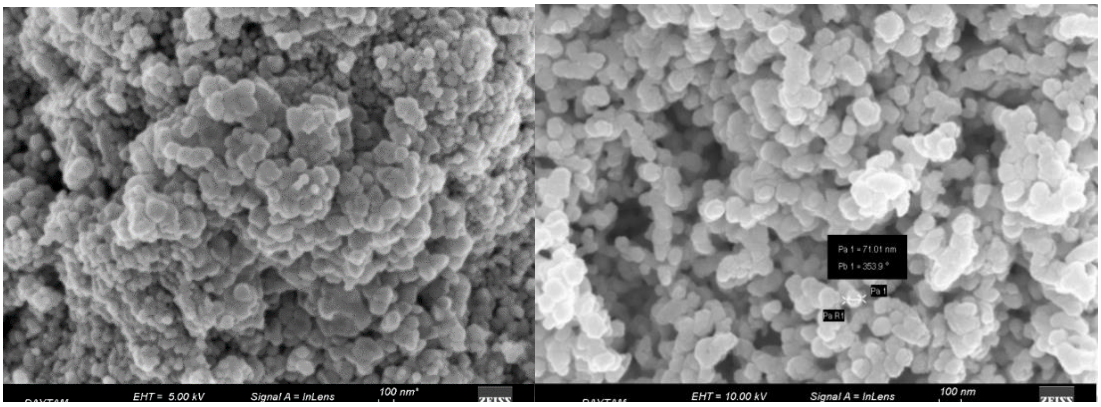

Fig. 4 (a, b). SEM images of the AgNPs.

3458, 2098, 1638 and 739 cm^{-1}. The shifts in the FTIR spectra of the bonds indicate that the AgNPs formed in the reaction are coated with grape vinegar. The wide and dense peak at 3280.9 cm^{-1} in the spectrum corresponds to the OH stretching vibrations of the phenol/carboxylic group present in the extract, the alkyne group present in the phyto-components in the plant structure showing the peak at 2093 cm^{-1}. The peak of the extract at 1608.8 cm^{-1} belongs to the C = O stretching or amide bending. Peak nitro N-O bending [26] at 1394.4 cm^{-1} refers to peaks of 678.9 and 837.1 cm^{-1} C-H alkenes. The findings are supported by some studies that synthesize AgNPs using plant extract [35-38].

Scanning electron microscopy analysis (SEM)

SEM device; it works on the basis of collecting and examining the interactions formed by dropping electrons originating from tungsten end onto the sample to be examined. In this way, the surface topography and structure of the sample examined are informed. SEM images, SEM images were taken to examine the morphology of silver nanoparticles synthesized by Green synthesis method and given in Fig. 4a and 4b. Silver NPs obtained as a result of SEM analysis were determined to have an average size of 20 nm (6-40 nm) and spherical shape [39].

X-ray diffraction

XRD analysis of Ag NPs obtained in Fig. 5 is

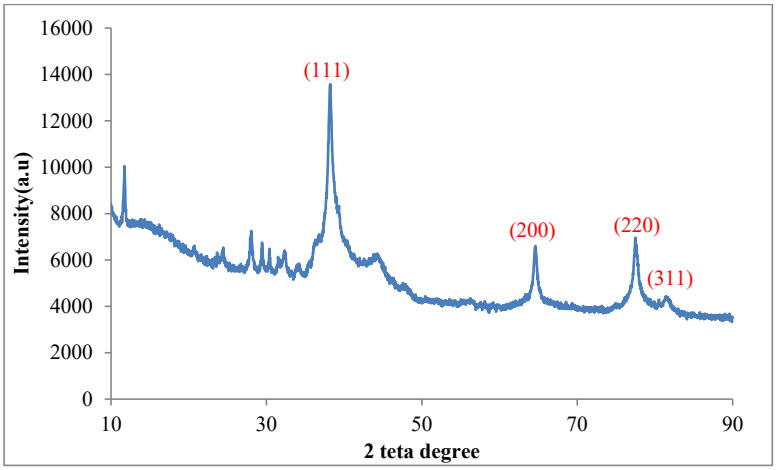

Fig. 5. XRD image of the AgNPs.

given. As a result of the analysis, a dense peak confirming the presence of silver nanoparticles obtained by green synthesis was determined. Fig. 5 shows the presence of silver nanoparticles at 25 ° C, which corresponds to the planes 38.15, 44.05 and 64.38, 77.32 corresponding to the three planes (111), (200), (220) and (311), respectively.

The obtained Ag NPs are compatible with literature and obtained with high efficiency [6].

Antimicrobial activity results

Silver NPs showed higher inhibition activity than other antibiotics (AMP10, SXT25 and K30) to the same degree (25 mm) as the antibiotic AMC30 in *M. luteus.* Again, the compound showed a lower inhibition effect in *S. aureus* than in the same antibiotics as the K30 antibiotic alone. The silver compound showed a higher inhibition effect in *B. cereus* than the antibiotic AMC30 (22 mm) and other antibiotics. The bacteria is known as an opportunist [40]. The silver compound showed a significantly higher inhibition activity (25 mm) than all standard antibiotics in *P. aeroginosa* (Table 1). The genus *Pseudomonas* is common in nature, leading to opportunistic and nosocomial infections. Among these agents, *P. aeruginosa* is one of the leading causes of nosocomial infections, it can develop resistance to various antibiotics and causes high mortality and morbidity due to infections [41,42]. *P. aeruginosa* is responsible for 10-25% of hospital infections [43,44]. Since *P. aeruginosa* usually shows multiple antibiotic resistances, it also causes problems in treatments. *P. aeruoginosa* septicemia occurs especially in weakened and immunosuppressed patients and has a mortality rate of 10-20%. In *K. pneumonia*, the silver compound had the same (20 mm) inhibition effect as the SXT25 antibiotic alone, but lowers than the others.

The compound also showed high inhibitory activity (21 mm) in *B. abortus* (Table 1). *Br. abortus* has gram-negative bacterium that causes premature abortion of cattle fetus [45], furthermore, it is a very serious, debilitating and sometimes chronic human pathogen that can affect various organs [28, 46].

Ag NPs showed higher inhibition effect against *S. typhi H* than only one standard antibiotic (AMP 10-11 mm) (15 mm) and lower inhibition than others (Table 1). *Salmonella serovars* cause many different clinical symptoms, ranging from asymptomatic infection to severe typhoid-like syndromes. [44-46]. The silver compound showed a higher inhibition effect than other antibiotics (AMP10, SXT25, AMC 30-10 mm, 18 mm, 14 mm respectively) except for the same antibiotic (K30-25mm) in *E. coli* (Table 1). It is a bacterium usually found in the lower intestines of warm-blooded organisms (endoderms). Many *E. coli* species are harmless, but some serotypes can cause serious food poisoning in humans. *E. coli* bacteria are normally live in the intestines of humans and animals. Most *E. coli* are harmless and in fact an important part of a healthy human intestinal system. However, some *E. coli* species are pathogenic, ie they may cause disease, diarrhea or disease outside the intestinal tract (Source: CDC-Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division

Table 1. Antimicrobial activities of AgNPs (diameter of zone of inhibition (mm)).

Microorganisms		Compound	Standart Antibiotics				
		Ag NPs	AMP 10*	SXT 25	AMC 30	K 30	NYS 100
Gram (+)	*M. luteus*	25	22	21	25	23	N
	S. epidermis	17	26	25	27	25	
	S. aureus	25	30	24	30	25	N
	B. cereus	22	23	25	20	28	N
Gram (-)	*P. aeroginosa*	25	8	18	15	14	N
	K pneumonia.	20	21	20	21	23	N
	B.abortus	21	-	-	-	-	N
	S.typhi H	15	11	17	19	20	N
	E.coli	25	10	18	14	25	N
	P. vulgaris	22	17	19	20	21	N
Maya	*C.albicans*	26	N	N	N	N	20

* Standard reagents (diameter of zone inhibition (mm). SXT25, sulfamethoxazole 25 µg; AMP10, Ampicillin 10 µg; NYS100, Nystatin 100 µg; K30, Kanamycin 30 µg; AMC30, Amoxycillin 30 µg. N: not tried.

Table 2. The frequencies of SCE and MN in human lymphocytes treated with different concentrations Ag NPs

Test Items	Concentrations	SCE/Cell ± S.E.	MN numbers ± S.E.
Control (-)		6.04 ± 0.12[a]	1.82 ± 0.09[a]
Control (+)	5 µM	9.24 ± 0.08[d]	3.48 ± 0.04[d]
Ag	5 µg/mL	6.62 ± 0.04[a]	2.10 ± 0.02[a]
Ag	10 µg/mL	7.06 ± 0.10[b]	2.24 ± 0.11[b]
Ag	20 µg/ mL	7.42 ± 0.03[b]	2.70 ± 0.15[bc]
Ag	40 µg/mL	8.04 ± 0.14[c]	2.92 ± 0.07[c]
Ag	80 µg/mL	8.32 ± 0.06[c]	3.15 ± 0.08[d]
Ag	160 µg/mL	8.95 ± 0.02[d]	3.26 ± 0.04[d]

Sodium azide (NaN_3) was used as positive controls for human peripheral lymphocytes.
[a, b,c,d] Statistically significant differences in the same column are indicated by the different superscripts (α = 0.05).

of Foodborne, Waterborne and Environmental Diseases (DFWED).

In addition, the compound showed significantly higher inhibition activity (22 mm) *in P. vulgaris* than all standard antibiotics (Table 1). *P. vulgaris* is a patient with weak immune systems.

Finally, the compound showed a higher (26 mm) inhibition effect in yeast, *C. albicans* (Table 1). Systemic fungal infections, including *C. albicans,* have emerged as significant causes of death and disease in immunocompromised patients (organ or ligament transplantation, adjuvants, cancer chemotherapy) [47–50]. As a result; the synthesized compound showed similar or higher inhibition effects in both Gram (+) and Gram (-) bacteria and yeast, similar to standard antibiotics and antifungals. At the same time, compared with standard antibiotics, the compound has an inhibitory effect against pathogenic bacteria and yeast (Table 1). In addition, it was determined that the synthesized compounds showed more inhibitory effect on gram-negative bacteria (Table 1).

Genotoxicity activitiy

The effects of genotoxicity for Ag NPs were investigated by SCE and MN tests. When the results obtained from the SCE and MN test systems are evaluated in Table 2, it is determined

that the silver nanoparticles increase the SCE and MN averages with genotoxic effect with increasing concentrations. It was found that the increase in the MN and SCE averages of 5 µg/mL in the concentrations used in the study was statistically insignificant and the highest genotoxic effect was in the 160 µg/mL application [51, 52].

When the results obtained from the SCE and MN test systems are evaluated in Table 1, it is determined that the silver nanoparticles increase the SCE and MN averages with genotoxic effect with increasing concentrations. It was found that the increase in the MN and SCE averages of 5 µg/mL in the concentrations used in the study was statistically insignificant and the highest genotoxic effect was in the 160 µg/mL application.

CONCLUSION

In this study, AgNPs were synthesized by green synthesis method and morphological and structural characterization was performed. Then, the genotoxic effects of AgNPs obtained *in vitro* were investigated. From the results obtained, it has shown that at known non-toxic concentrations of AgNPs synthesized by the non-toxic green method [52, 53], it can certainly compete with, or even better result, commercial antibiotics used in the treatment of microbial infections and open wounds.

Therefore, it is believed that the resulting environmentally friendly silver nanoparticles can be used as an excellent antimicrobial agent against pathogenic microorganisms or as an additive to antimicrobial products.

ACKNOWLEDGEMENTS

This work was carried out with support of Research Development Center of Ataturk University, Turkey by a project numbered FAD-2018-6321.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

REFERENCES

1. De Gaetano F., Ambrosio L., Raucci M.G., Catauro M., (2005), Sol-gel processing of drug delivery materials and release kinetics. *J. Mater. Sci. Mater. Med.* 16: 261–265.
2. Zhao G., Stevens E., (1998), Multiple parameters for the comprehensive evaluation of the susceptibility of *Escherichia coli* to the Ag-NPs ion. *Biometals*. 11: 27–32.
3. Devaux X., Laurent C., Rousset A., (1993), Chemical synthesis of metal nanoparticles dispersed in alumina. *Nanostruc. Mater.* 2: 339–346.
4. Karaduman I., Güngör A. A., Nadaroğlu A., Altundas A., Acar S., (2017), Green synthesis of γ-Fe_2O_3 nanoparticles for methane gas sensing. *J. Mater. Sci. Mater. Electron.* 28: 16094–16105.
5. Nadaroglu H., Ince S., Gungor A. A., (2017), Green synthesis of gold nanoparticles using quail egg yolk and investigation of potential application areas. *Green Process Synth.* Doi:10.1515/gps-2016-0091.
6. Nadaroglu H., Gungor A. A., Ince S., Babagil A., (2017), Green synthesis and characterisation of platinum nanoparticles using quail egg yolk. *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.* 5: 43-47.
7. Demirci Gültekin D., Alaylı Güngör A.,Önem H., Babagil A., Nadaroglu H., (2017), Synthesis of copper nanoparticles using a different method: Determination of its antioxidant and antimicrobial activity. *J. Turkish Chem. Soc. Sect. A Chem.* 3: 623-636.
8. Singhal G., Bhavesh R., Kasariya K., Sharma A. R., Singh R. P., (2011), Biosynthesis of silver nanoparticles using Ocimum sanctum (Tulsi) leaf extract and screening its antimicrobial activity. *J. Nanoparticules Res.* 13: 2981–2988.
9. Mukherjee P., Ahmad A., Mandal D., Senapati S., Sainkar S. R., Khan M. I., Parischa R., Ajaykumar P. V., Alam M., Kumar R., Sastry M., (2001), Fungus-mediated synthesis of Ag-NPs nanoparticles and their immobilization in the mycelial matrix: A novel biological approach to nanoparticle synthesis. *Nano Lett.* 1: 515–519.
10. Spring S., Schleifer K-H., (1995), Diversity of magnetotactic bacteria. *Syst. Appl. Microbiol.* 18: 147-153.
11. Jiang H, Manolache S., Wong A. C. L., Denes F. S., (2004), Plasma-enhanced deposition of Ag-NPs nanoparticles onto polymer and metal surfaces for the generation of antimicrobial characteristics. *J. Appl. Polym. Sci.* 93: 1411–1422.
12. Duran N., Marcato P. D., Alves O. L., Souza G. I. H. D., (2005), Mechanistic aspects of biosynthesis of Ag-NPs nanoparticles by several Fusarium oxysporum strains. *J. Nanobiotech.* 3: 8-12.
13. Becker R. O., (1999), Ag-NPs ions in the treatment of local infections. *Met. Based Drugs*. 6: 311–314.
14. Klaus T., Olsson E., (1999), Ag-NPs -based crystalline nanoparticles, microbially fabricated. *Proc. Natl. Acad. Sci.* 96: 13611–13614.
15. Patlolla A. K., Hackett D., Tchounwou P. B., (2015), Genotoxicity study of silver nanoparticles in bone marrow cells of Sprague-Dawley rats. *Food Chem. Toxicol.* 85: 52–60.
16. Kim Y. S., Kim J. S., Cho H. S., Rha D. S., Kim J. M., Park J. D., Choi B. S., Lim R., Chang H. K., Chung Y. H., Kwon I. H., Jeong J., Han B. S., Yu I. J., (2008), Twenty-eight-day oral toxicity, genotoxicity, and gender-related tissue distribution of silver nanoparticles in Sprague-Dawley rats. *Inhal. Toxicol.* 20: 575–583.
17. Foldbjerg R., Dang D. A., Autrup H., (2011), Cytotoxicity and genotoxicity of silver nanoparticles in the human lung cancer cell line A549. *Arch. Toxicol.* 85: 743–750.
18. Fenech, M., (2000), The *in vitro* micronucleus technique. *Mutat. Res. Mol. Mech. Mutagen.* 455: 81–95.
19. Orhan F., Çeker S., Anar M., Agar G., Arasoglu T., Gulluce

M., (2016), Protective effects of three luteolin derivatives on aflatoxin B 1-induced genotoxicity on human blood cells. *Med. Chem. Res.* 25: 2567–2577.
20. Anar M., Aslan A., Ceker S., Kızıl H. E., Alpsoy L., Agar G., (2015), Determination of antigenotoxic effects of four lichen species by using human lymphocytes. *Fresenius Environ. Bull.* 24: 4251–4256.
21. Perry P., Evans H. J., (1975), Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange. *Nature.* 258: 121–125.
22. Ceker S., Orhan F., Kizil H. E., Alpsoy L., Agar G., Aslan A., Agar G., (2015), Genotoxic and antigenotoxic potentials of two Usnea species. *Toxicol Indust. Heal*. 31: 990-999.
23. Stick H. F., Dunn B. P., (1986), Relationship between cellular levels of beta-carotene and sensitivity to genotoxic agents. *Int. J. Cancer*. 38: 713–717.
24. Schillinger U., Lucke F. K., (1989), Antimicrobial activity of *Lactobacillus sake* isolated from meat. *Appl. Environ. Microbiol.* 55: 1901–1906.
25. Kolhe N. H., Jadhav S. S., (2018), Synthesis, characterization and biological activity of mixed ligands complexes of quinolin-8-ol and substituted chromones with Mn(II), Co(II), Ni(II) and Cu(II) metal ions. *Res. Chem. Intermed.* 45: 973–996.
26. Almeida C. G. de, Reis S. G., Almeida A. M. de, Diniz C. G., da Silva V. L., Le Hyaric M., (2011), Synthesis and antibacterial activity of aromaticand heteroaromatic amino alcohols. *Chem. Biol. Drug Des.* 78: 876–880.
27. Nithya C., Gnanalakshmi B., Pandian S. K., (2011), Assessment and characterization of heavy metal resistance in Palk Bay sediment bacteria. *Mar. Environ. Res.* 71: 283–294.
28. Nartop D., Özkan E. H., Gündem M., Ceker S., Agar G., Ogutcu H., Sari N., (2019), Synthesis, antimicrobial and antimutagenic effects of novel polymeric-Schiff bases including indol. *J. Mol. Struct.* 1195: 877–882.
29. Magaldi S., Mata-Essayag S., Hartung De Capriles C., Perez C., Colella M. T., Olaizola C., Ontiveros Y., (2004), Well diffusion for antifungal susceptibility testing. *Int. J. Infect. Dis.* 8: 39–45.
30. Balouiri M., Sadiki M., Ibnsouda S. K., (2016), Methods for *in vitro* evaluating antimicrobial activity: A review. *J. Pharm. Anal.* 6: 71–79.
31. Valgas C., Souza S. M. De, Smânia E. F. A., Smania A., (2007), Screening methods to determine antibacterial activity of natural products. *Braz. J. Microbiol.* 38: 369–380.
32. Xiang Y., Liu X., Mao C., Liu X., Cui Z., Yang X., Yeung K. W. K., Zheng Y., Wu S., (2018), Infection-prevention on Ti implants by controlled drug release from folic acid/ZnO quantum dots sealed titania nanotubes. *Mater. Sci. Eng. C.* 85: 214–224.
33. Zhu Y., Liu X., Yeung K. W. K., Chu P. K., Wu S., (2017), Biofunctionalization of carbon nanotubes/chitosan hybrids on Ti implants by atom layer deposited ZnO nanostructures. *Appl. Surf. Sci.* 400: 14–23.
34. Mao C., Xiang Y., Liu X., Cui Z., Yang X., Yeung K. W. K., Pan H., Wang X., Chu P. K., Wu S., (2017), Photo-Inspired antibacterial activity and wound healing acceleration by hydrogel embedded with Ag/Ag@AgCl/ZnO nanostructures. *ACS Nano.* 11: 9010–9021.
35. Cicek S., Gungor A. A., Adiguzel A., Nadaroglu H., (2015), Biochemical evaluation and green synthesis of nano silver using peroxidase from Euphorbia (*Euphorbia amygdaloides*) and its antibacterial activity. *J. Chem.* 2015: Article ID: 486948, 7 pages.
36. Padalia H., Moteriya P., Chanda S., (2015), Green synthesis of silver nanoparticles from marigold flower and its synergistic antimicrobial potential. *Arab. J. Chem.* 8: 732–741.
37. Kokila T., Ramesh P. S., Geetha D., (2015), Biosynthesis of silver nanoparticles from Cavendish banana peel extract and its antibacterial and free radical scavenging assay: A novel biological approach. *Appl. Nanosci.* 5: 911–920.
38. Ghotekar S., Savale A., Pansambal S., (2018), Phytofabrication of fluorescent silver nanoparticles from Leucaena leucocephala L. leaves and their biological activities. *J Water Environ. Nanotechnol*. 3: 95–105.
39. Roy A., Bulut O., Some S., Mandal A. K., Yilmaz M. D., (2019), Green synthesis of silver nanoparticles: biomolecule-nanoparticle organizations targeting antimicrobial activity. *RSC Adv*. 9: 2673–2702.
40. Miller J. M., Hair J. G., Hebert M., Hebert L., Robertz F. J. Jr., Weyant R. S., (1997), Fulminating bacteremia and pneumonia due to Bacillus cereus.[erratum appears in J Clin Microbiol 1997 May;35(5):1294]. *J. Clin. Microbiol.* 35: 504–507.
41. Pollack M., (1995), *Pseudomonas aeruginosa.*Mandell G, Bennett J, Dolin R, (eds).: *Principles and Practice of Infectious Disease*. 1980–2003.
42. Hanberger H., Garcia Rodriguez J. A., Gobernado M., Goossens H., Nilsson L.E., Struelens M. J., (1999), Antibiotic susceptibility among aerobic gram-negative bacilli in intensive care units in 5 European countries. *French Port ICU Study Groups JAMA*. 281: 67–71.
43. Gunseren F., (1999), A surveillance study of antimicrobial resistance of gram-negative bacteria isolated from intensive care units in eight hospitals in Turkey. *J. Antimicrob. Chemother.* 43: 373–378.
44. Çetin Ç. B., Yalçın A. N., Turgut H., Cetin B., Tefci F., (1999), Hastane infeksiyonlarında antibiyotik maliyeti. *Hastan. İnfeksiyon. Derg.* 3: 161–164.
45. Halling S. M., Peterson-Burch B. D., Bricker B. J., Zuerner R .L., Qing Z., Li L. L., Kapur V., Alt D. P., Olsen S. C., (2005), Completion of the genome sequence of Brucella abortus and comparison to the highly similar genomes of Brucella melitensis and *Brucella suis. J. Bacteriol.* 187: 2715–2726.
46. Sauret J. M., Vilissova N., (2002), Human brucellosis. *J Am. Board Fam. Pract*. 15: 401–406.
47. Ogutcu H., Yetim N. K., Özkan E. H., Eren O., Kaya G., Sari N., Disli A., (2017), Nanospheres caped Pt(II) and Pt (IV): Synthesis and evaluation as antimicrobial and Antifungal Agent. *Polish J. Chem. Technol.* 19: 74–80.
48. Koçoğlu S., Ogutcu H., Hayvalı Z., (2019), Photophysical and antimicrobial properties of new double-armed benzo-15-crown-5 ligands and complexes. *Res. Chem. Intermed.* 45: 2403–2427.
49. Altundas A., Erdogan Y., Ögütcü H., Kizil H. E., Agar G., (2016), Synthesis and In-vitro Antimicrobial and Anti-mutagenic Activities of some Novel 2-(2-Hydroxybenzylideneamino)-5,7-dihydro-4H-thieno[2,3-c]pyran-3-carbonitrile Derivatives. *Fresenius Environ. Bull*. 25: 5411–5418.
50. Ceker S., Ogutcu H., Meral S., Agar A. A., Agar G., (2019), Synthesis, Anti-microbial and anti-mutagenic activities of some schiff bases derivatives containing thiophene group. 32: 2679-2686.
51. Wise J. P., Goodalea B. C., Wisea S. S., Craig G. A., Pongan

A. F., Walter R. B., Thompson W. D., Ng A. K., Aboueissa A. M., Mitani H., Spalding M.J., Mason M. D., (2017), Silver nanospheres are cytotoxic and genotoxic to fish cells. *J. Autism. Dev. Disord.* 47: 549–562.

52. Özkan E. H., Kizil H. E., Şakiyan İ., Nartop D., Sari N., Agar G., (2018), Anti-genotoxic Effects of Schiff bases and their Mn (III) Complexes Containing L-Aspartic acid and L-Phenylalanine. *Gazi Univ. J. Sci.* 31: 408-414.
53. Bangale S., Ghotekar S., (2019), Bio-fabrication of silver nanoparticles using rosa *Chinensis L.* extract for antibacterial activities. *Int. J. Nano Dimens*. 10: 217–224.
54. Ghotekar S., Pansambal S., Pawar S. P., Pagar T., Oza R., Bangale S., (2019), Biological activities of biogenically synthesized fluorescent silver nanoparticles using *Acanthospermum hispidum* leaves extract. *SN Appl. Sci*. 1: 1342-1348.