Nanomed Res J 7(3):254-263, Summer 2022

RESEARCH ARTICLE

Ag-ZnO nanoparticles: synthesis, characterization, antibacterial activity on S. mutans, along with cytotoxic effect on U87 cell line

Armita Forutan Mirhosseini[1], Hadi jabbari[2], Alireza Nouralishahi[3], Zahra Bahman[4], Sana Yahyazadeh Jasour[5], Afsaneh Nekouei[6*]

[1] Dentist, Student Research Committee, School of Dentistry, Kerman University of Medical Sciences, Kerman, Iran

[2] Department of Chemistry, Payame Noor University, P.O. Box 19395-4697 Tehran, Iran

[3] Isfahan Eye Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

[4] Faculty of dentistry,Belarusian state medical university,Belarus,minsk

[5]Department of Microbiology, School of Biology, College of Science, University of Tehran, Tehran, Iran

[6] Assistant professor Dentistry facility Shahr -e -kord University of medical science

ARTICLE INFO

Article History:
Received 16 May 2022
Accepted 27 Jul 2022
Published 01 Aug 2022

Keywords:
Ag-dop-ZnO NP
Green synthesis
cytotoxic
antibacterial activity

ABSTRACT

In here, we introduced a rapid and green synthesis route to prepare pure zinc oxide (ZnO) and silver-doped zinc oxide (Ag-dop-ZnO) nanoparticles (NP) using Prosopis *farcta* extract. The physic-chemical property of the synthesized NPs was identified using PXRD, FESEM, FT-IR, UV-Vis, and EDX devices. According to the obtained outcomes of PXRD, UV-Vis, as well as EDX analysis, Ag was well doping into the ZnO structure. Moreover, spherical shape was observed with a mean size of 25-40 nm based on FESEM outcomes for the synthesized nanoparticles. The cytotoxic effect of the synthesized nanoparticles was assessed on glioblastoma (U87) cell line using an MTT test. The cytotoxic outcomes presented that the doped NPs have a more cytotoxic effect on the U87 cell line. The antibacterial activity of synthesized NP was studied against *Streptococcus mutans* bacterium through disk diffusion and microdilu¬tion method. Based on the outcomes of microdilu¬tion and disk diffusion assay and of the synthesized NP, Ag-dop-ZnO NP shows higher antibacterial activity against *Streptococcus mutans* than the pure NPs. Hence, the synthesized NPs can be suggested for disinfectant products and cancer treatment.

How to cite this article
Forutan Mirhosseini A., jabbari H., Nouralishahi A., Bahman Z., Yahyazadeh Jasour S., Nekouei A. Ag-ZnO nanoparticles: synthesis, characterization, antibacterial activity on S. mutans, along with cytotoxic effect on U87 cell line. Nanomed Res J, 2022; 7(3): 254-263. DOI: 10.22034/nmrj.2022.03.005

INTRODUCTION

Zinc oxide nanoparticles have multiple applications as a result of their upper surface-to-volume ratio, non-toxic, wide bandgap, and cheapness [1]. They are used in solar cells, electronics, sensors, and biochemical assays. According to recent studies, ZnO NP have antibacterial, anti-inflammatory, antifungal, antiviral, and anti-cancer properties. The antimicrobial properties of this metal oxide are caused by the production of hydrogen peroxide from the surface of nanoparticles, thus preventing the growth of bacteria through damage to DNA and destruction of the cell wall. For this reason, compared to other metal oxides, these nanoparticles are used as disinfectants in the health, food, and cosmetics industries[1, 2].

Tooth decay is recognized an infectious microbial disease, which results in breakdown and destruction of calcified tooth tissue[3]. This degradation process is resultant from the activity of carbohydrate-fermenting bacteria, the production of acid, and the subsequent demineralization of tooth tissue[4]. Tooth decay is reliable to be an infectious illness of microbial origination

* Corresponding Author Email: *afsaneh.nekouei@yahoo.com*

caused by many types of bacteria in the mouth. *Streptococcus mutans* is important bacteria in causing dental caries[5]. Therefore, it is reasonable to use substances that can kill or destroy this type of bacteria in dental materials to prevent and control this disease. Production and development of dental materials with antimicrobial properties has been one of the most important and major goals of dental science and dentistry for many years[5, 6]. Several studies and experiments have been done in this regard, on substances with antibacterial properties to treat oral infections. Among these materials are ZnO NP. For example, Hernández-Sierra *et al.* studied the antibacterial activity of ZnO on *Streptococcus mutants* and showed that these nanoparticles have an antibacterial effect on this strain[7].

Gliomas are a group of tumors of the central nervous system (CNS), which is resultant from the abnormal division of neuroglia cells[8]. Eighty percent of primary malign brain tumors are caused by gliomas. Glioblastoma is the most popular and acute type of malignant brain tumors in adults. It is a type of astrocytic cell tumor with histological symptoms such as increased mitosis, polymorphism, endothelial proliferation, and rapid necrosis. The methods used to treat acute glioma are determined by the type, location, and degree of the tumor, and the general health of the patient. Among these methods are surgery and removal of the tumor, radiation therapy, the use of antiepileptic drugs and corticosteroids, and chemotherapy with DNA alkylation agents such as temozolomide[9]. Treatment of glioblastoma leads to poor treatment and recurrences owing to the presence of a blood-brain barrier that prevents the entrance of chemical drugs to the brain and the recurrence of the tumor. This is caused by the self-renewal of glioma stem cells. This factor reduces the average survival of patients with glioblastoma. The drugs have some side effects and these treatments are time-consuming, costly, and ineffective. Hence, it is essential to look for new methods and strategies to prevent the disease[10, 11].

Nanomaterials have a key function in membrane and DNA destruction as well as suddenly cell death through creating lipid peroxidation and oxidative stress. Hence, effective therapies can be achieved leading to tumor destruction through decreasing therapy side effects [12, 13]. Among nanocomposites, ZnO is known as a biocompatible substance that results in cytotoxicity by causing an inflammatory effect and the formation of superoxide, H_2O_2, and free hydroxyl radicals in mammalian cells. Increased free radicals in the cellular environment, due to oxidative stress, activate the necrosis and apoptosis pathways and eventually cell death[14].

ZnO NP are prepared with different morphologies with rod, prismatic, ring, spherical, and flower-like shapes. Also, different methods are used to make these nanoparticles, including hydrothermal, laser evaporation, sol-gel, combustion, vapor deposition, and microwave. The green method is an easy ad fast method to produce oxide nanoparticles[15]. The green synthesis method, which uses environmentally friendly compounds and microorganisms, has been accepted as a promising method to minimize the limitations of chemical and physical methods. This method uses plant extracts, fungi, bacteria, and other microorganisms. In recent years, extensive efforts were made to use plant extracts to make various metal oxides and metal nanoparticles as a result of their low cost and environmental compatibility[16-18].

Prosopis farcta is a member of the *leguminosa* family, native to regions of the Americas, Africa, and Asia. This plant has several therapeutic effects, most of which are due to the presence of corticosteroids, vitexin, tryptamine, tannins, and epigenetics in this plant. Among the properties of this plant are the improvement of shortness of breath, stomach ulcers, bloody diarrhea, rheumatism, an increase in HDL cholesterol and decrease in LDL cholesterol, and a decrease in blood glucose concentration[19, 20]. Due to the compounds in this plant, it was selected for the synthesis of these nanoparticles. So, in this study, pure ZnO and Ag-dop-ZnO NP by applying the aqueous extract of *Prosopis farcta* were prepared, and studied their antibacterial and cytotoxic performance.

EXPERIMENTAL SECTION

Extraction of Prosopis farcta

The *Prosopis farcta* powder weighed, and distilled water with a 1:10 ratio added to it. It was shaken to 15 h at 150 rpm, and then it filtered. The obtained extract was applied for the next tests.

Synthesis of pure and Ag-dop-ZnO NP

For synthesis of pure and doped ZnO NP, 10 mL of extract was volumed up to 100 mL using distilled water. Then, it was positioned in a 70 ˚C

water bath. About 100 mL of the solutions including $Zn(NO_3)_2.9H_2O$ (Merck) as well as $Ag(NO_3)$ (Merck) was prepared following the formula of $Ag_{1-x}Zn_xO$ whenever the added percentages of $Ag(NO_3)$ were 0, and 3, respectively. In the next step, the solutions dried in 90 ˚C for 10 hours. Finally, the dried powder was calcined in a 600 ˚C furnace for 1.30 h.

Characterization of Nanoparticles

The physic-chemical property of ZnO and Ag-dop-ZnO NP was specified through UV-Vis device with Rayleighuv-2100 model made in Chine, PXRD device with PANalyticalX'Pert PRO model, Cu Kα, made in the Netherlands, and FESEM microcopy with MIRA3 TESCAN model made in Czech.

Antibacterial performance

Disk diffusion test

Antibacterial activity of ZnO and Ag-dop-ZnO NP tested through the disk diffusion assay on *Streptococcus mutants* (ATCC: 35668) bacteria based on NCCLS 2000 standard. The bacterial suspension was adjusted to 0.5 McFarland to achieved 1.5×10^8 colony-forming units/mL. The surface of Brain Heart Infusion (BHI) agar inoculated with the bacterial suspension was placed on inoculated agars and then 6 mm discs were impregnated with 20 µL of the NP. Plates were incubated at 37 ˚C for 24 h. Chloramphenicol (CP) and Ciprofloxacin (CF) were applied as the antibacterial standard against the pathogen. The zones of inhibition and their values were presented on mean (Table 1).

MIC and MBC tests

Minimum inhibitory concentration (MIC) of pure ZnO and 3% Ag-dop-ZnO NP were tested through the microdilution test using 96 well plates. In this test, Chloramphenicol (CP) and Ciprofloxacin (CF) were as positive control, and distilled water was a negative control. The nanoparticles diluted to 100 µL of Brain Heart Infusion broth. Then, 100 µL of the bacteria was added to each well, as well as the microplates taken at 37 °C for 24 h. The MICs were considered as the lowest concentration presenting the visible growth of the bacteria. The minimum bactericidal concentration (MBC) was tested by using a sub-cultured of 100 µL of solution from each well of plates on Brain Heart Infusion (BHI) agar plates. It was then incubated at 37 °C for 24 h, which were considered as lowest concentration of the synthesized samples from bacterial growth.

Cytotoxicity Test

Cell culture

Here, brain glioblastoma cells (U87) were used to survive the cytotoxicity of the synthesized NPs. U87 cells were achieved from the Pasteur Institute of Iran. The cells were transferred to Falcon tubes, as well as then centrifuged at 833 rpm for 9 min. DMEM as culture medium was applied for cell culture. To each culture medium, 10% fetal bovine serum (FBS), 100 µg/mL of streptomycin, and 100 international units/mL of penicillin were added to prevent microbial growth. To grow the cells, the plates including culture medium was incubated under condition of 5% CO_2 at 37 °C.

MTT test

MTT test was applied to study cytotoxicity of synthesized NP. The cytotoxicity effect of each of the two nanoparticles was tested in 1, 5, 10, 50, 100, and 500 µg/mL of concentrations. In the first, cell suspension of 10^4 cells per well was added to each of the 96-well plate wells, and incubated to 24 hours. After ensuring that the cells adhered to the floor of the plate, the culture medium was drained from each well of the 96-wells plate. 100 µL from nanoparticles was added to wells. Then, the plate was incubated for 24 hours under condition of 5% CO_2 at 37 ˚C. Then, 10 microliters of MTT solution added to each well and the plate was re-incubated for 4 hours. Finally, the adsorption of each sample was read through an ELISA reader at a wavelength of 490 nm and the percentage of cell viability (survival) was measured through Equation 1.

Cell viability (%) = [100 × (sample abs)/ (control abs)] Eq. 1

Table 1. Mean inhibition zone and standard deviation for pure ZnO and Ag-dop-ZnO NP.

Nanoparticles	Inhibition zone (mm)			
	bacteria	Control negative	Control positive	
	S. mutans	Water	CP	CF
ZnO	10 ± 0.3	NA	20 ± 0.3	9 ± 0.1
Ag-dop-ZnO	14 ± 0.3	NA	20 ± 0.3	9 ± 0.1

NA= not appearing.

RESULTS AND DISCUSSION

PXRD analysis

The PXRD patterns of the ZnO and 3% Ag-dop-ZnO NP are presented in Fig. 1. The pattern of pure ZnO nanoparticles depicted diffraction peaks at 2θ= 31.56, 34.63, 36.49, 47.76, 56.23, 62.12, 66.78, 67.15, 69.20, 72.05, and 76.92˚. This is accordance with wurtzite structure of ZnO (JCPDS card: 36-1451) [21]. The pattern of the doped NPs showed some additional diffraction peaks located in 2θ = 37.14, 44.86, and 64.42˚ associated with the face-centered cubic (FCC) phase of metallic silver (ICSD card: 52362)[20]. The crystallite size was identified by using Scherrer's equation [22, 23], which was achieved as 35 nm and 19 nm for ZnO and 3% Ag-dop-ZnO NP. It can be expressed that doping of silver ions leads to increasing the crystal size of doped samples because of the ionic radius of silver (0.126 Å) Compared with the zinc (0.74 Å).

FESEM/EDX/ PSA analysis

The FESEM images of ZnO and 3% Ag-dop-ZnO NP were depicted based on their morphology and particle size. As shown in Fig. 2, the particle size of pure ZnO was observed as about 40 nm. The particle size of doped NP was decreased with the doping of silver into the crystalline lattice of zinc oxide. According to the PSA curve, the particle size distribution of pure and 3% Ag-dop-ZnO NP was obtained as 37.69 and 22.58 nm. The EDX outcomes of synthesized NPs demonstrated the satisfying entrance of silver into the ZnO structure. EDX data shown that weight percent of zinc and oxygen elements was 32.43, and 67.57 for pure NP, and it was 62.17, 2.34, and 35.49 for zinc, silver, and oxygen elements in 3% Ag-dop-ZnO NP, which agreed to the absence of any impurity at NP (Fig. 3).

UV-Vis analysis

UV-Vis becomes an attractive tool for identifying, and studying nanomaterials. Fig. 4 displays the electron spectra of synthesized ZnO and 3% Ag-dop-ZnO NP. The maximum absorption peaks appeared in ranges of 378 and 372 nm for pure ZnO and 3% Ag-dop-ZnO NP. As seen in Figure 4, the absorption peak of ZnO NP was shifted to the lower wavelength as blue shift due to the decrement in crystallization and quantum confinement phenomena. The lack of another absorption peak overall the electron spectra demonstrated the lack of impurities in the nanoparticles, as well as approved their satisfying optical properties[21].

FT-IR analysis

The FT-IR spectra of synthesized ZnO and 3% Ag-dop-ZnO NP were obtained in range of 400–4000 (Fig. 5). The absorption band in range of 3450 cm^{-1} indicated the strong stretching vibration of OH groups of adsorbed H_2O upon NP surface. Absorption band in range of 1627 cm^{-1} belongs to the stretching vibration of the C-H groups. The absorption band at range of 476 cm^{-1} is related to the vibrational band Zn-O in ZnO NP graph, which was shifted to 471 cm^{-1} for FT-IR spectrum of Ag-dop-ZnO NP. It showed that intensity of vibrational

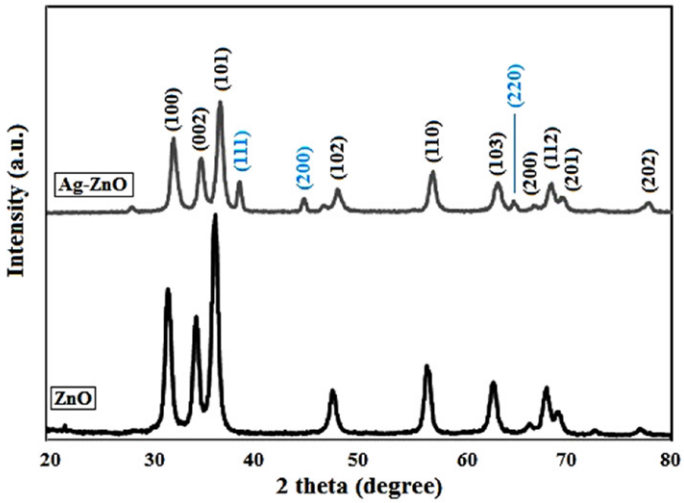

Fig. 1. PXRD graphs of ZnO and 3% Ag-dop-ZnO NP.

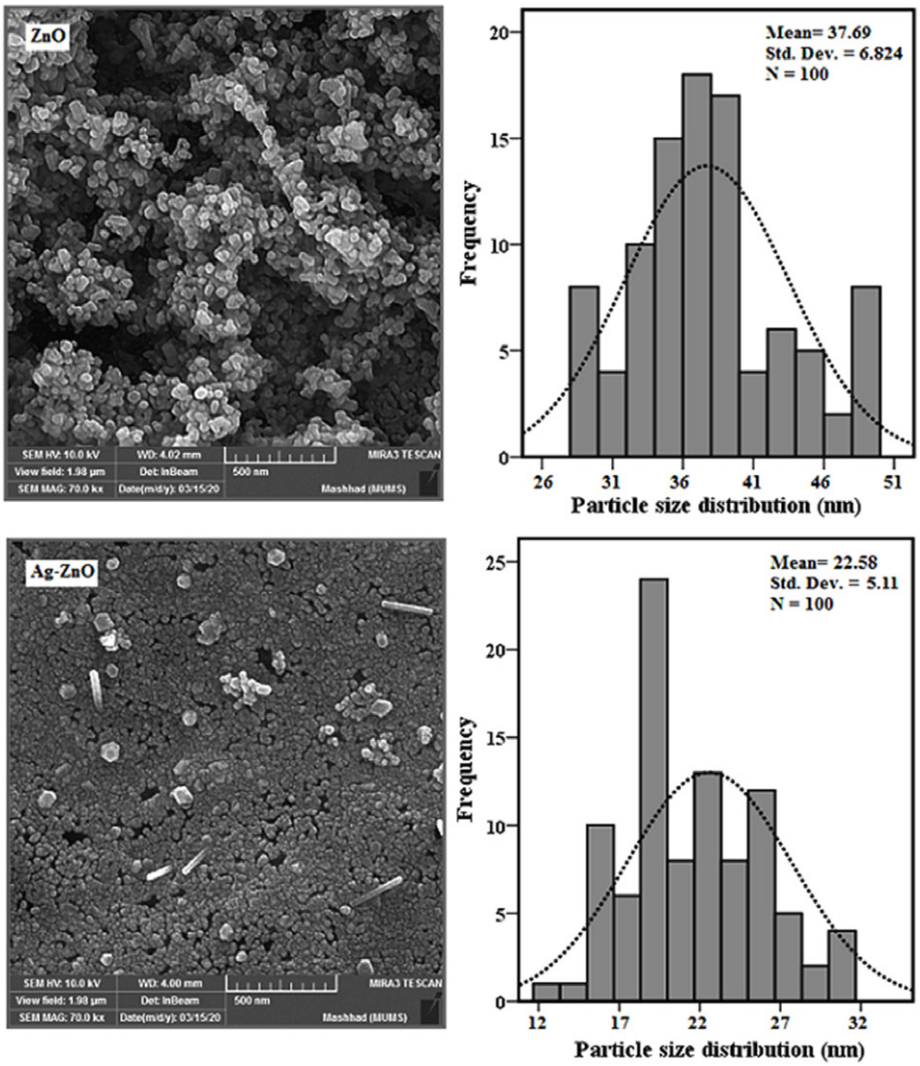

Fig. 2. FESM images of ZnO and 3% Ag-dop-ZnO NP.

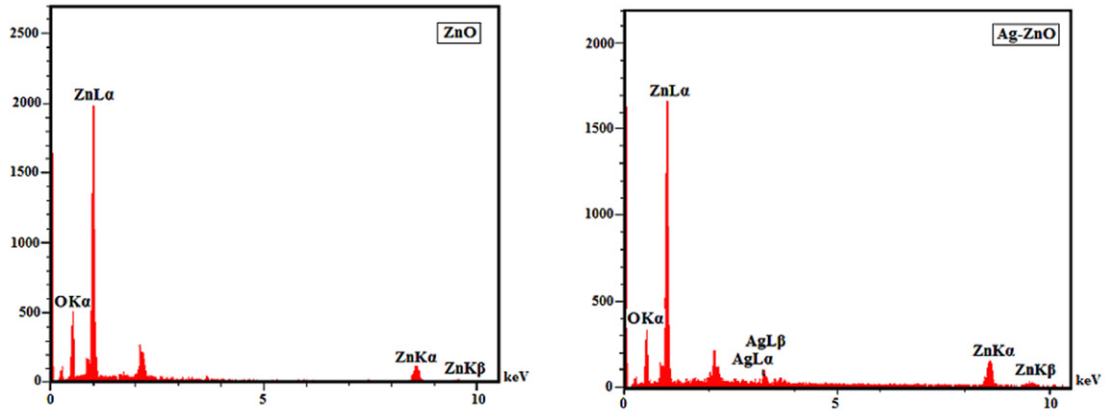

Fig. 3. EDX graphs of ZnO and 3% Ag-dop-ZnO NP.

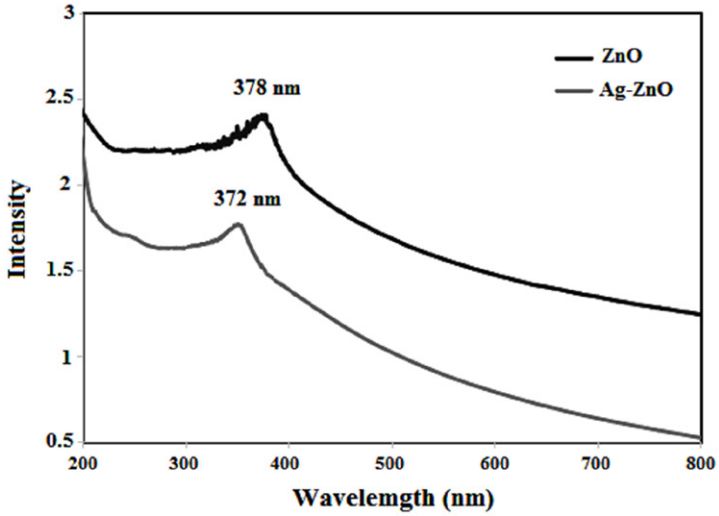

Fig. 4. UV-Vis spectra of ZnO and 3% Ag-dop-ZnO NP.

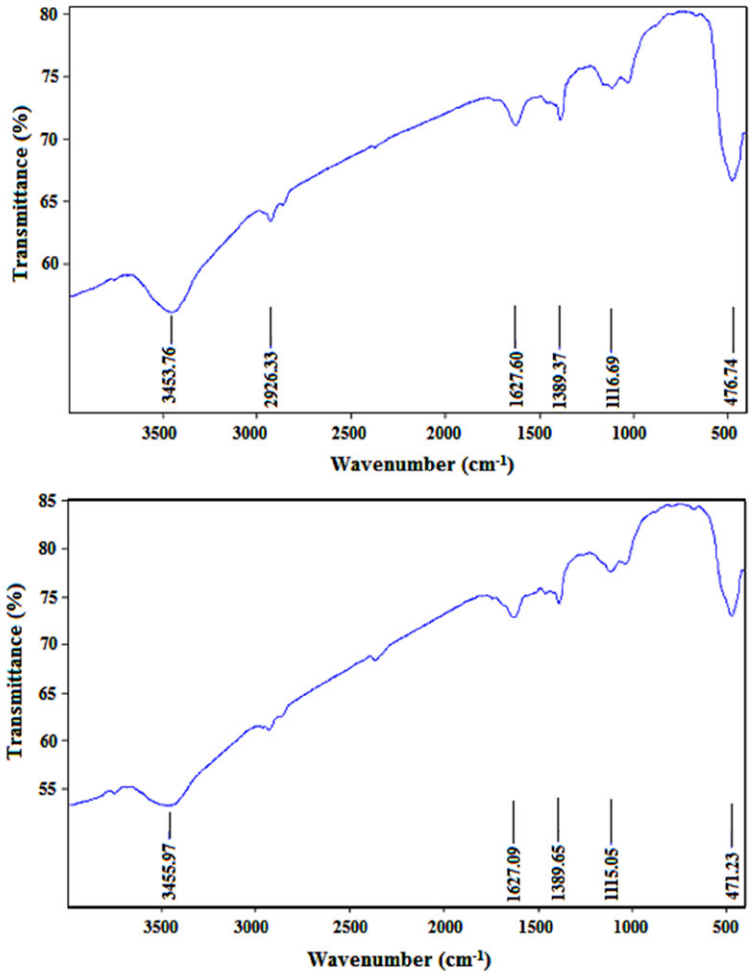

Fig. 5. FT-IR spectra of ZnO and 3% Ag-dop-ZnO NP.

band of Zn-O was decreased with the decrease in particle size of Ag-dop-ZnO NP[21].

Antibacterial activity

Antibacterial activity of ZnO and Ag-dop-ZnO NP survey by using disk diffusion and microdilution assay against *Streptococcus mutans* bacteria. The inhibition zone of disks from diffusion of nanoparticles was displayed in Fig. 6. As seen in Figure 6, Ag-dop-ZnO NP have an inhibition zone larger than ZnO NP, which can be due to silver doped to ZnO structure.

The gained data from MIC assay of synthesized ZnO and Ag-dop-ZnO NP were provided at 250 and 125 µg/mL on *S. mutans* bacteria, respectively (Fig. 7). The obtained data from MBC of synthesized nanoparticles were provided in Table 2. The outcomes showed that MBC of Ag-dop-ZnO NP against *S. mutans* was 250 µg/mL, and also there was no outcome shown for *S. aureus* bacteria.

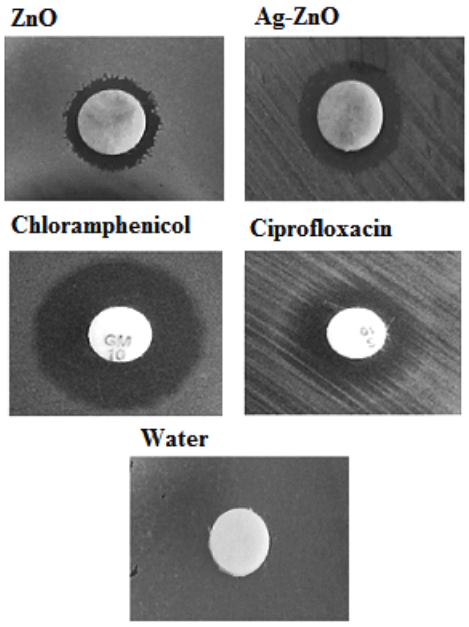

Fig. 6. Antibacterial effect of ZnO and 3% Ag-dop-ZnO NP against *S. mutans*

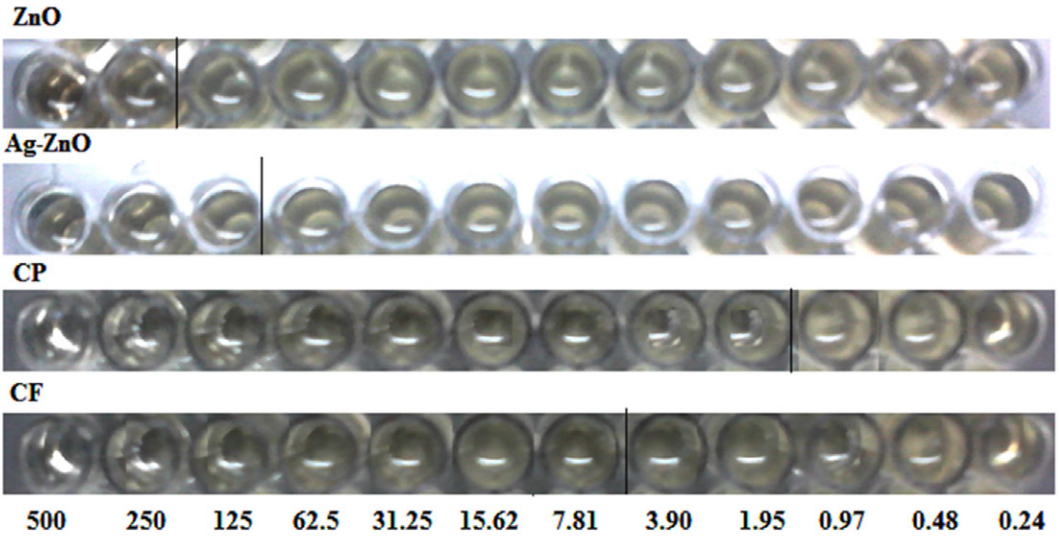

Fig. 7. Minimum inhibitory concentration of ZnO and 3% Ag-dop-ZnO NP on *S. mutans*

According to gained outcome, the synthesized Ag-dop-ZnO NP not only inhibited the growth of *S. mutans* but also killed them. A study by Nigussie *et al.* stated that Ag doping to ZnO NP caused the increase of antibacterial performance on pathogenic bacteria likes *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*[24].

The results showed that silver doping has more significant negative effects than zinc oxide nanoparticles. This may be because silver destabilizes the plasma membrane potential, resulting in a decrease in adenosine triphosphate levels inside the cell, which kills bacteria by targeting the cell membrane. In fact, silver break down the inhibitory components in the outer membrane of the bacterium, which in turn causes the exponential release of molecules such as liposaccharides and purines from the cytoplasmic membrane [20]. So, it suggested that synthesized Ag-dop-ZnO NP could be utilized as effective ingredient in treatment of oral infections.

Cytotoxic effect

In recent years, the strategy of using nanoparticles as a carrier system for drug treatment and delivery has made significant progress. One of these nanoparticles that have attracted the attention of researchers is zinc oxide nanoparticles, which in recent years have been used as a treatment agent for several diseases, including cancer in vivo and in vitro. Zinc oxide nanoparticles are metal oxide nanoparticles that are toxic to cancer cells and inhibit cell invasion and sensitization to radiation and chemotherapy, protecting reactive oxygen species (ROS) and increasing apoptosis in cells[19].

In this study, the cytotoxic effect of ZnO and Ag-dop-ZnO NP at different concentrations in glioblastoma (U87) cell line has been reviewed. The finding of this study showed that doping Ag to ZnO NP was incremented the toxicity activity on U87 cells than to ZnO NP. The toxicity effect of pure ZnO NP was similar to the effect of doxorubicin as a control at the 500 µg/mL concentration (Fig. 8). The IC50 was achieved for ZnO and Ag-dop-ZnO NP were obtained at >1000 and 270.4 mg/L. Ag-dop-ZnO NP had a greater inhibitory activity compared to ZnO NP.

In general, as nanoparticle concentrations increase, more nanoparticles enter the cell and induce its toxic activity through creating oxidative

Table 2 Minimum inhibition and bactericidal concentrations of ZnO and 3% Ag-dop-ZnO NP.

Samples	*S. mutans*	
	MIC (µg/mL)	MBC (µg/mL)
ZnO	250	-*
Ag-dop-ZnO	125	250
Chloramphenicol	1.95	3.90
Ciprofloxacin	3.90	15.62

*means no antibacterial effect.

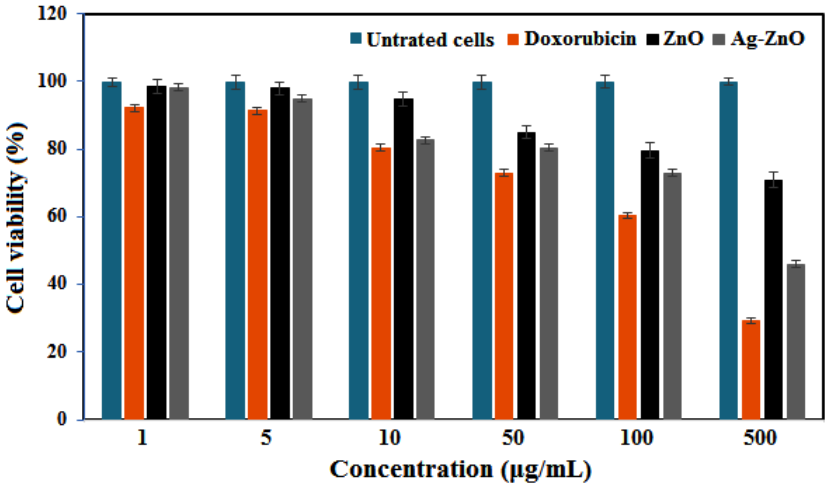

Fig. 8. Cell viability of ZnO and 3% Ag-dop-ZnO NP on U87 cell line after 24 hours incubation

stress within the cell. This oxidative stress causes double-stranded DNA failures and thus the death of cancer cells[25]. Various studies have been carried out for survey the cytotoxic activity of zinc oxide nanoparticles in various cell lines.

R. Wahab *et al*. showed that ZnO nanostructures have a cytotoxic effect on human glioma (U87) cells. According to their results, ZnO NP increased mitotic death and interphase death of the U87 cells [26]. Our results presented that Ag-dop-ZnO NP had a more toxic effect on U87 cells than to ZnO NP. However, the doping of silver was accompanied by an increase in the toxicity of zinc oxide nanoparticles. Therefore, doped nanoparticles showed a better anti-cancer effect against U87 cells.

CONCLUSION

In this study, an easy, inexpensive, and green method was provided to prepare ZnO and Ag-dop-ZnO NP by using leaf extract of *Prosopis farcta*. The FESEM image presented the spherical shape of synthesized nanoparticles. The FESEM and other characterization results of nanoparticles presented that doping silver decreased the size of zinc oxide particles. The cytotoxic effect of the synthesized NP was investigated on glioblastoma (U87) cell line by utilized an MTT test. Then, the antibacterial performance of pure and Ag-dop-ZnO NP was assessed against *Streptococcus mutans*. The results of both assays showed that the doped silver caused the increased effectiveness of cytotoxic and antibacterial performance of ZnO NP. Hence, the synthesized NP can be suggested as an effective ingredient in the treatment of oral infections and cancer treatment.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

REFERENCES

1. Sirelkhatim A, Mahmud S, Seeni A, Kaus NHM, Ann LC, Bakhori SKM, et al. Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. Nano-micro letters, 2015;7(3):219-42. https://doi.org/10.1007/s40820-015-0040-x
2. Mosleh-Shirazi S, Kouhbanani MAJ, Beheshtkhoo N, Kasaee SR, Jangjou A, Izadpanah P, et al. Biosynthesis, simulation, and characterization of Ag/Ag-FeO2 core-shell nanocomposites for antimicrobial applications. Applied Physics A, 2021;127(11):1-8. https://doi.org/10.1007/s00339-021-05005-7
3. Lovegrove JM. Dental plaque revisited: bacteria associated with periodontal disease. J N Z Soc Periodontol. 2004;(87):7-21.
PMID: 15143484
4. Larsen T, Fiehn NE. Dental biofilm infections-an update. Apmis, 2017;125(4):376-84. https://doi.org/10.1111/apm.12688
5. Zhang Y, Fang J, Yang J, Gao X, Dong L, Zheng X, et al. Streptococcus mutans-associated bacteria in dental plaque of severe early childhood caries. Journal of oral microbiology, 2022;14(1):2046309. https://doi.org/10.1080/20002297.2022.2046309
6. Ramburrun P, Pringle NA, Dube A, Adam RZ, D'Souza S, Aucamp M. Recent advances in the development of antimicrobial and antifouling biocompatible materials for dental applications. Materials, 2021;14(12):3167. https://doi.org/10.3390/ma14123167
7. Hernández-Sierra JF, Ruiz F, Pena DCC, Martínez-Gutiérrez F, Martínez AE, Guillén AdJP, et al. The antimicrobial sensitivity of Streptococcus mutans to nanoparticles of silver, zinc oxide, and gold. Nanomedicine: Nanotechnology, Biology and Medicine, 2008;4(3):237-40. https://doi.org/10.1016/j.nano.2008.04.005
8. Choi SA, Kwak PA, Park C-K, Wang K-C, Phi JH, Lee JY, et al. A novel histone deacetylase inhibitor, CKD5, has potent anti-cancer effects in glioblastoma. Oncotarget, 2017;8(6):9123. https://doi.org/10.18632/oncotarget.13265
9. Thon N, Thorsteinsdottir J, Eigenbrod S, Schüller U, Lutz J, Kreth S, et al. Outcome in unresectable glioblastoma: MGMT promoter methylation makes the difference. Journal of neurology, 2017;264(2):350-8. https://doi.org/10.1007/s00415-016-8355-1
10. Bhowmik A, Khan R, Ghosh MK. Blood brain barrier: a challenge for effectual therapy of brain tumors. BioMed research international, 2015;2015. https://doi.org/10.1155/2015/320941
11. Mehrabian A, Vakili-Ghartavol R, Mashreghi M, Saremi SS, Badiee A, Arabi L, et al. Preparation, characterization, and biodistribution of glutathione PEGylated nanoliposomal doxorubicin for brain drug delivery with a post-insertion approach. Iranian Journal of Basic Medical Sciences, 2022;25(3):302. https://doi.org/10.21203/rs.3.rs-742064/v1
12. Fu PP, Xia Q, Hwang H-M, Ray PC, Yu H. Mechanisms of nanotoxicity: generation of reactive oxygen species. Journal of food and drug analysis, 2014;22(1):64-75. https://doi.org/10.1016/j.jfda.2014.01.005
13. Yu Z, Li Q, Wang J, Yu Y, Wang Y, Zhou Q, et al. Reactive oxygen species-related nanoparticle toxicity in the biomedical field. Nanoscale research letters, 2020;15(1):1-14. https://doi.org/10.1186/s11671-020-03344-7
14. Bisht G, Rayamajhi S. ZnO nanoparticles: a promising anticancer agent. Nanobiomedicine, 2016;3(Godište 2016):3-9. https://doi.org/10.5772/63437
15. Kołodziejczak-Radzimska A, Jesionowski T. Zinc oxide-from synthesis to application: a review. Materials, 2014;7(4):2833-81.https://doi.org/10.3390/ma7042833
16. Sarani M, Tosan F, Hasani SA, Barani M, Adeli-Sardou M, Khosravani M, et al. Study of in vitro cytotoxic performance of biosynthesized α-Bi2O3 NPs, Mn-doped and Zn-doped Bi2O3 NPs against MCF-7 and HUVEC cell lines. Journal

of Materials Research and Technology, 2022;19:140-50. https://doi.org/10.1016/j.jmrt.2022.05.002
17. Kouhbanani MAJ, Sadeghipour Y, Sarani M, Sefidgar E, Ilkhani S, Amani AM, et al. The inhibitory role of synthesized Nickel oxide nanoparticles against Hep-G2, MCF-7, and HT-29 cell lines: the inhibitory role of NiO NPs against Hep-G2, MCF-7, and HT-29 cell lines. Green Chemistry Letters and Reviews, 2021;14(3):444-54. https://doi.org/10.1080/17518253.2021.1939435
18. Miri A, Najafzadeh H, Darroudi M, Miri MJ, Kouhbanani MAJ, Sarani M. Iron oxide nanoparticles: biosynthesis, magnetic behavior, cytotoxic effect. ChemistryOpen, 2021;10(3):327-33. https://doi.org/10.1002/open.202000186
19. Miri A, Khatami M, Ebrahimy O, Sarani M. Cytotoxic and antifungal studies of biosynthesized zinc oxide nanoparticles using extract of Prosopis farcta fruit. Green Chemistry Letters and Reviews, 2020;13(1):27-33. https://doi.org/10.1080/17518253.2020.1717005
20. Miri A, Sarani M. Biological studies of synthesized silver nanoparticles using Prosopis farcta. Molecular biology reports, 2018;45(6):1621-6. https://doi.org/10.1007/s11033-018-4299-0
21. Hamidian K, Sarani M, Barani M, Khakbaz F. Cytotoxic performance of green synthesized Ag and Mg dual doped ZnO NPs using Salvadora persica extract against MDA-MB-231 and MCF-10 cells. Arabian Journal of Chemistry, 2022;15(5):103792. https://doi.org/10.1016/j.arabjc.2022.103792
22. Nazaripour E, Mousazadeh F, Moghadam MD, Najafi K, Borhani F, Sarani M, et al. Biosynthesis of lead oxide and cerium oxide nanoparticles and their cytotoxic activities against colon cancer cell line. Inorganic Chemistry Communications, 2021;131:108800. https://doi.org/10.1016/j.inoche.2021.108800
23. Cao Y, Alijani HQ, Khatami M, Bagheri-Baravati F, Iravani S, Sharifi F. K-doped ZnO nanostructures: Biosynthesis and parasiticidal application. Journal of Materials Research and Technology, 2021;15:5445-51. https://doi.org/10.1016/j.jmrt.2021.10.137
24. Nigussie GY, Tesfamariam GM, Tegegne BM, Weldemichel YA, Gebreab TW, Gebrehiwot DG, et al. Antibacterial activity of Ag-doped TiO2 and Ag-doped ZnO nanoparticles. International Journal of Photoenergy, 2018;2018. https://doi.org/10.1155/2018/5927485
25. Jia P, Dai C, Cao P, Sun D, Ouyang R, Miao Y. The role of reactive oxygen species in tumor treatment. RSC advances, 2020;10(13):7740-50. https://doi.org/10.1039/C9RA10539E
26. Wahab R, Kaushik N, Khan F, Kaushik NK, Choi EH, Musarrat J, et al. Self-styled ZnO nanostructures promotes the cancer cell damage and supresses the epithelial phenotype of glioblastoma. Scientific reports, 2016;6(1):1-13. https://doi.org/10.1038/srep19950