Synergistic Activities of Tetracycline with CuO Nanoparticles Study of its Activity on Antibiotic-Resistant Bacteria

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Abstract

The synergy between antibiotics and nanoparticles is one of the most significant ways for combating antibiotic resistance. Tetracycline/copper oxide nanoparticles were produced utilizing a chemical precipitation approach and a physical process including ultrasound in this research. Tetracycline nanoparticles were mixed with CuO nanoparticles. The findings demonstrated a consistent distribution of copper oxide nanoparticles in the tetracycline particle matrix. The resulting nanocomposites were characterized using XRD, EDX, and SEM techniques (CuO) and matched with the (ICDD) database of the International Center for X-ray Diffraction [ICDD card no. 41-0254] The average crystal size was 44.83 nm. The average crystal size was 22.37 nm. The TCS with CuO synergy was studied. By assessing the lowest inhibitory concentration of two types of bacteria using ELISA equipment, the antibacterial compatibility and synergistic studies of tetracycline/ CuO nanoparticles were compared to normal tetracycline particles acquired from Samarra Pharmaceutical Laboratory. The MIC of synergistic TCs with CuO NPs against Staphylococcus was 32 µg/ml, indicating high efficacy in killing germs. In comparison, the MIC of standard TCs was 512 µg/ ml, indicating an increase in efficacy of the above compound compared to standard TCs. While the synergistic TCs with CuO NPs did not change the MIC concentration towards pseudomonas aeruginosa compared to the standard TCs. Hemolysis was also used to investigate the toxicity of the compound (TCs + CuO NPs). The results of the tests revealed that the chemicals were safe. It is considered non-toxic at all doses of TCs + CuO NPs because it did not break up the platelet and had no harmful effect.

Keywords: Tetracycline, CuO Nanoparticles, Synergistic, Antibiotic-Resistant

1. Introduction

Tetracyclines are yellow crystalline compounds formed from Hydro naphthalene, which contains four hexagonal rings as the main structure and are abbreviated as (TCS) and have water solubility and low solubility in alcohol, and are insoluble in organic solvents [1]. Its molecular weight is 444.435 g/mol. It's also a broad-spectrum antibiotic, with activity against a variety of gram-positive and gram-negative bacteria [2]. Antimicrobial resistance is a global issue that has a negative impact on human health. Bacterial resistance to antibiotics is one of the most serious worldwide health and economic issues, forcing researchers to look into developing new antibiotics to combat resistant bacterial strains. The time of treatment is lengthened as a result of resistant microorganisms. Furthermore, it raises the risk of sickness [3]. Reduced cell membrane permeability, development of efflux systems, synthesis of antibiotic-inactivated enzymes, and alteration of target molecules are all strategies used by bacteria to build antibiotic resistance [4]. As for mutations, they occur randomly and relatively slowly in bacterial assemblies, and bacteria can acquire genetic material from resistant bacterial species present with them in the same place through gene transfer [5]. Accordingly, bacteria can develop antibiotic resistance genes [6]. Several types of antibiotic resistance exist, including the Multi-Drug Resistant, which resists at least three antibiotics from three different groups. The XDR Drug-Resistant is extensively resistant to at least one of all groups, and the Pan-Drug Resistant. Finally, this type of resistance to almost all classes of antibiotics is a global phenomenon that has made treating many diseases complex [7]. This is a consequence of the inappropriate and indiscriminate use of antibiotics [8]. One of the resistant bacterial species found in abundance in Iraq was within the subject of the research sample, Pseudomonas aeruginosa and Staphylococcus aureus. These bacteria can grow and survive under simple growth conditions, which helps them stay on surfaces and medical equipment for a long time, thus causing infections. Where it causes severe infections for hospitalized patients or people with weak immune systems, and the condition with this bacteria is often complex due to its ability to penetrate and multiply in tissues, so it is responsible for many acute and chronic diseases and infections in humans, such as chronic lung inflammation in patients Cystic fibrosis, meningitis, dermatitis, soft tissues, diabetic foot, eye inflammation, bone, joint inflammation, urinary tract infections, enteritis, bacteremia in patients with severe burns and ear infections [9, 10].Staphylococcus aureus can cause many diseases such as minor skin infections such as blisters, meningitis and soft tissues, endocarditis, osteomyelitis, arthritis, recurrent tonsillitis, bacteremia, and pneumonia, sinusitis, and food poisoning [10]. The treatment of S. aureus has become more difficult, as it is considered one

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of the most important types of bacteria that are resistant to antibiotics, as it is resistant to a large group of them, such as its resistance to beta-lactam and Vancomycin, which was used as an alternative to beta-lactam antibiotics [11], tetracycline, chloramphenicol, methicillin, erythromycin, trimethoprim, and others. of antigens [12, 13]. Cases of increasing antibiotic resistance pose an ongoing threat, especially when the number of new antibiotics is minimal [14, 15]. This scarcity has exacerbated the problem of resistance, necessitating the development of alternative approaches such as nano-antibiotics (nAbts), which are based on the physicochemical coupling of nanoparticles with antibiotics or artificially pure antibiotic molecules, with at least one of their dimensions not exceeding 100 nm and are one of the most promising applications of nanotechnology [16, 17]. It has devised novel ways for reconstituting antibacterial compounds that are already in use. Antibiotics coupled with NPs or pure antibiotics developed at the nanoscale can penetrate bacterial cell membrane barriers with greater precision and stability than ordinary antibiotic molecules [18]. Because of their unusual physical, chemical, optical, and mechanical features, inorganic nanoparticles have gotten a lot of interest. Nanomaterials are a subset of nanomaterials. the CuO nanomaterials come first because of their surface catalytic effect and chelating effect. At the same time, other traditional metal oxides only have a catalytic impact on the surface, which leads to an oxidation reaction [19]. Metal oxide nanomaterials can be manufactured with large surface areas; It is more suitable for selective and multifunctional biological applications [20, 21]. CuO has exciting properties such as low toxicity, good thermal and chemical stability, low cost, high catalytic reusability, ease of handling, and electronic bonding effects [22, 23]. CuO NPs have shown their activities as antibacterial and antifungal agents, as cell membranes have pores at the nanometer scale [24]. As a result, the proposed mechanism of action is based on the fact that produced NPs are smaller than cell membrane holes, allowing them to penetrate them and form stable complexes containing key enzymes within cells, causing cellular performance to be hampered and bacteria to die [25].

2. Materials and Methods

The chemicals used in this study, $CuCl_2.2H_2O$, ethanol is 99%Indian, NaOH 97% Indian, HCl 99%Indian, Tetracycline 99.9%Pharmaceutical Company Samarra Iraq, Nutrient agar& Muller Hinton broth from HIMEDLA(INDIA), Bacterial isolates (Pseudomonas aeruginosa, Staphylococcus aureus) were obtained and diagnosed from Baquba Teaching Hospital, Iraq.

Synthesis of CuO Nanoparticles

A concentration of 0.25M of$CuCl_2.2\ H_2O$. The $CuCl_2$ solution was prepared by dissolving 1 gm in 25 ml ethanol, and a concentration of 0.5M of 1.6 gm in 80 ml ethanol was designed, where the NaOH solution was prepared by continuous distillation and added to the $CuCl_2$ solution with constant stirring using a magnetic stirrer at room temperature. The solution was neutralized for 30 minutes with drops of weak hydrochloric acid at PH8, then filtered, rinsed with water, and allowed to dry at room temperature before being burned for 3 hours at 700 °C, yielding a powdery black precipitate.

Synergistic TCS with CuO Nanoparticles

In 100 ml of deionized water, 0.4 gm of CuO NPs were dissolved, and the response was 2 gm of tetracycline in 100 ml. It was added to CuO NPs for two hours at room temperature with constant stirring by a magnetic stirrer, then placed in an ultrasonic for three hours, and then dried in a 45 °C oven.

Minimal - Inhibitory Concentration

Multiple sequence concentrations of antagonist (TCs + CuO NPs) ranging from (16 - 32 - 64 - 128 - 256 - 512- 1024µg/ml) were generated by adding varying proportions of this antagonist from their prepared safe solutions and transferring (100 L) of deionized water to the plate pits. Each bacterial isolate was divided into three replicates using a polystyrene plate with 96 holes, with (100 µL) of the antibody distributed in the holes with the highest concentration of 1024 except for the holes containing the control is water and cultured bacteria. Shifts were made by (100 µL) of the counter-pit with a concentration of 1024 being transferred to the next hole. Similarly, the transfer from one hole to the other was accomplished. By reaching the last hole, we discarded (100 µL), and the bacteria were distributed in the amount of (100 µL) for each hole in the plate. The plate was covered and incubated in the incubator at 37 ° c [26] before being measured using an ELISA reader at 630 nm [27].

Hemolysis assay

To identify dangerous or non-toxic substances, the hemolysis assay was used to screen for nano-antibiotics at various concentrations (16, 32, 64, 128, 256, 500 µg/ml). Plasma The plasma layer is removed from the cells, and the cells are washed numerous times with PBS, each time adding 1 ml of PBS and centrifuged for 2 minutes to separate the cells from the PBS. The blood cell suspension was made by taking 1 mL of the cells after they had been washed numerous times and mixing it with 9ML PBS. The antibody is introduced at a varied concentration in each tube with a volume of (1200µL). It's mixed with 300µL of the cell suspension to make a final volume of 1.5 ml, then incubated for two hours in the incubator before being separated by centrifuge equipment at 2000 rpm for two minutes. Then there was the rate of hemolysis [26].

3. Results and Discussion

Characterization by X-ray diffraction

Figure 1shows XRD, and the prepared X-ray spectrum of copper oxide was matched with the standard spectrum of (CuO) with the (ICDD) database of the International Center for X-ray diffraction [ICDD card no. 41-0254] The value of the average crystal size was (44.83 nm), and this is consistent with the literature [28, 29]. Figure 2 shows that the XRD and the synergistic TCS spectra were matched to CuO and the mean crystal size was 22.37 nm.

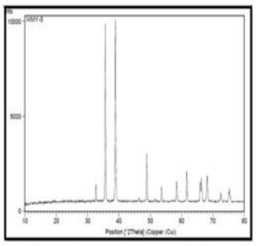

Figure1 X-ray diffraction spectrum for CuO

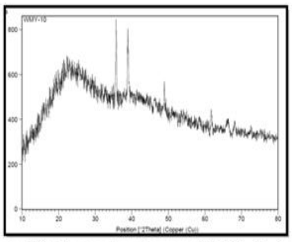

Figure 2 X-ray diffraction spectrum for synergistic TCS with CuO

Characterization by energy-dispersive X-rays

The proportion of elements present in CuO NPs was diagnosed by energy dispersive X-ray, as shown in Figure 3. The results showed the presence of copper at (86.6%) and oxygen at (13.2 %), respectively, so a high purity copper oxide nanomaterial appeared. As for the percentage of elements present in the synergistic TCs with NPs CuO by dispersive energy X-ray, as shown in Figure 4, the results showed the presence of carbon (50.3%), copper (27.7%), oxygen (18.9%) and nitrogen (3.1%), and the loaded TCs showed It has high purity CuO NPs.

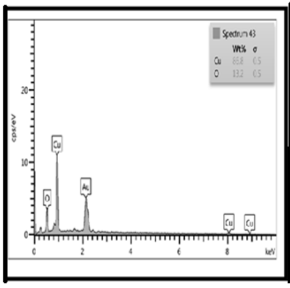

Figure 3 EDX spectrum of CuO

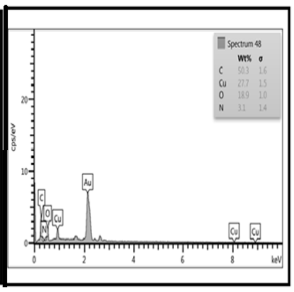

Figure 4 EDX spectrum of TCs with CuO

Characterization by scanning electron microscope

The morphological and structural compositions of CuO NPs were studied using an SEM scanning electron microscope. Figure 5 shows that the nanoparticles were prepared in the nanometer range, and the SEM images showed that some of the nanoparticles separated well from each other. At the same time, most of them were present in a lumpy form due to the electrostatic effects. This is consistent with behavior similar to the agglomeration of nanoparticles in previous studies, and the average diameter of these nanoparticles is 63.54 nm [30]. As for the ratio of morphological and structural structures synergistic TCs with NPs CuO, Figure 6 shows that the nanoparticles were prepared in the nanometer range. The SEM images showed that some nanoparticles separated well from each other. At the same time, most of them were present in a lumpy form due to the electrostatic effects, and this is consistent with the behavior that is similar to the agglomeration of nanoparticles in previous studies. The average diameter of these nanoparticles is 141.29 nm

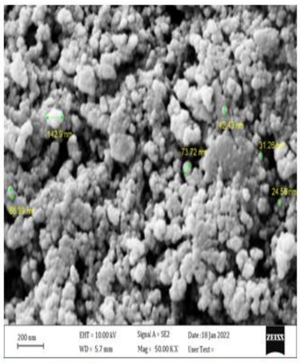

Figure 5 SEM image of CuO NPs

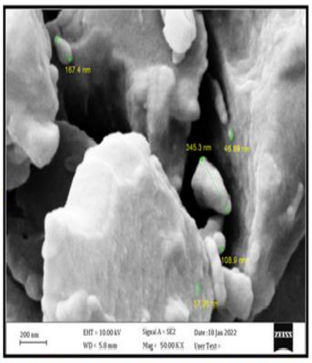

Figure 6 SEM image of synergistic TCs with CuO NPs.

Isolating Bacteria

The isolates used in this investigation were collected from Baquba Teaching Hospital's laboratory and clinical sources. They were diagnosed, and vitek® 2 compacts were used to determine their antibiotic resistance. The results showed that the first isolate was Pseudomonas aeruginosa was resistant to the antibiotics (Cefotaxime, Amikacin, Gentamicin, Ticarcillin-Clavulanate, Piperacillin, Cefepime, Ciprofloxacin, Tobramycin, Ceftazidime, Levofloxacin, Polymyxin, Meropenem). And the second isolate was Staphylococcus aureus resistant to the antigens Levocin, Staphylacin, Benzylcom, Staphylacin Occupil Gentamicin, Tobramycin, or Linezolid, Teicoplanin, Tetracycline, Tigecycline.

Determination of Minimal Inhibitory Concentration

The resistant bacterial isolates under study were tested to determine the minimum inhibitory concentration of (TCs, TCs +CuO NPs) compounds. The MIC was determined by the multiplicative serial concentration method (16 -32-64-128-256-512-1024µg/ml) with Middle Mueller Hinton Broth to take the test. The results in Table 1 showed high efficacy in killing bacteria. The MIC of the synergistic TCs with CuO NPs towards Staphylococcus aureus was 32 µg /ml. In comparison, the MIC of the standard TCs was 512 µg ml, which indicates an increase in the effectiveness of the above compound compared with the standard TCs. While the synergistic TCs with CuO NPs did not show any change in the MIC concentration towards pseudomonas aeruginosa compared to the standard TCs, which indicates the absence of any development in the anti-bacterial activity of this compound.

Table 1 Minimal Inhibitory Concentration of (TCs, TCs +CuO NPs)

compounds	Pseudomonas aeruginosa MIC µg/ml	Staphylococcus aureus MIC µg /ml
TCs	512	512
TCs+ CuO NPs	512	32

Determination Hemolysis assay

Hemolysis was tested to determine the compound's toxicity (TCs + CuO NPs), as the test results showed the safety of the compounds. It is considered non-toxic for all concentrations of the compound TCs + CuO NPs, which did not show any toxic effect through the absence of breakage in platelets, as shown in Tables 2 and Fig. 7

Table 2 Hemolysis assay to determine the compound's toxicity (TCs + CuO NPs)

TCs + CuO NPs	32	-
	64	-
	128	-
	500	-

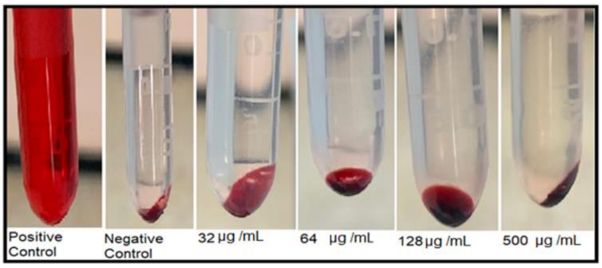

Figure 7 Hemolysis test for anti-TCs + CuO NPs

The anti-TCs + CuO NPs with it have more chance of eradicating bacteria. Since the valency of tetracycline-bound to copper has been reported in previous studies [31], this association may lead to an increase in the antibiotic effect on the bacterial membrane and the inability of the bacteria to resist the contact of nano-antibiotics. These limits could justify the synergistic property of our compound. Furthermore, nanoparticles appear to be more adsorbed on the surface of bacteria. It also revealed that Gram-positive bacteria are more sensitive than Gram-negative bacteria. This is also in line with past research. CuO NPs stabilize for the first time in this study, and their involvement in a better synergistic action with nanostructured antibiotics is explored. The findings of this study are particularly relevant since the procedures used to isolate the resistant bacteria will become sensitive to the bacteria themselves. Antibiotic nanoparticles' toxicity is linked to the production of reactive oxygen species (ROS) or the stimulation of pro-inflammatory mediators. When reactive oxygen species (ROS) are produced, nanoparticles destroy DNA, proteins, and cell membranes [32].

4. Conclusion

Chemical precipitation and a physical approach using ultrasound were used to make tetracycline/copper oxide nanoparticles. Tetracycline microparticles were mixed with copper oxide nanoparticles. The findings demonstrated a consistent distribution of copper oxide nanoparticles in the tetracycline particle matrix. By measuring the minimum inhibitory concentration of two bacteria types using an ELISA device, the antibacterial and blood compatibility of tetracycline/copper oxide nanoparticles, as well as synergistic studies, were compared to standard tetracycline particles obtained from Samarra Pharmaceutical Laboratory. The production of reactive oxygen species (ROS) has been explored as a major source of nanotoxicity and cell death, as well as having a greater synergistic effect on Tetracycline's antibacterial activity than normal antibiotics.

References

1. Dai Y, Liu M, Li J, Yang S, Sun Y, Sun Q, Wang W, Lu L, Zhang K, Xu J. A review on pollution situation and treatment methods of tetracycline in groundwater. Separation Science and Technology. 2020;55(5):1005-21. https://doi.org/10.1080/01496395.2019.1577445

2. Fuoco D. Classification framework and chemical biology of tetracycline-structure-based drugs. Antibiotics. 2012;1(1):1. https://doi.org/10.3390%2Fantibiotics1010001

3. Collignon P, Beggs JJ, Walsh TR, Gandra S, Laxminarayan R. Anthropological and socioeconomic factors contributing to global antimicrobial resistance: a univariate and multivariable analysis. The Lancet Planetary Health. 2018;2(9):e398-e405. https://doi.org/10.1016/S2542-5196(18)30186-4

4. Mubeen B, Ansar AN, Rasool R, Ullah I, Imam SS, Alshehri S, Ghoneim MM, Alzarea SI, Nadeem MS, Kazmi I. Nanotechnology as a Novel Approach in Combating Microbes Providing an Alternative to Antibiotics. Antibiotics. 2021;10(12):1473. https://doi.org/10.3390/antibiotics10121473

5. Thomas CM, Nielsen KM. Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nature reviews microbiology. 2005;3(9):711-21. Available from: https://www.nature.com/articles/nrmicro1234

6. Berglund F, Böhm M-E, Martinsson A, Ebmeyer S, Österlund T, Johnning A, Larsson DJ, Kristiansson E. Comprehensive screening of genomic and metagenomic data reveals a large diversity of tetracycline resistance genes. Microbial genomics. 2020;6(11). https://doi.org/10.1099%2Fmgen.0.000455

7. Ayaz M, Subhan F, Sadiq A, Ullah F, Ahmed J, Sewell RDE. Cellular efflux transporters and the potential role of natural products in combating efflux mediated drug resistance. Frontiers in Bioscience. 2017;22(4):732-56. https://orca.cardiff.ac.uk/id/eprint/98462

8. Ayaz M, Subhan F, Ahmed J, Khan A-u, Ullah F, Ullah I, Ali G, Syed N-i-H, Hussain S. Sertraline enhances the activity of antimicrobial agents against pathogens of clinical relevance. Journal of Biological Research-Thessaloniki. 2015;22(1):1-8. https://doi.org/10.1186/s40709-015-0028-1

9. Sivanmaliappan TS, Sevanan M. Antimicrobial susceptibility patterns of Pseudomonas aeruginosa from diabetes patients with foot ulcers. International journal of microbiology. 2011;2011. https://doi.org/10.1155/2011/605195

10. Chen AI, Dolben EF, Okegbe C, Harty CE, Golub Y, Thao S, Ha DG, Willger SD, O'Toole GA, Harwood CS. Candida albicans ethanol stimulates Pseudomonas aeruginosa WspR-controlled biofilm formation as part of a cyclic relationship involving phenazines. PLoS pathogens. 2014;10(10):e1004480. https://doi.org/10.1371/journal.ppat.1004480

11. Papp-Wallace KM, Endimiani A, Taracila MA, Bonomo RA. Carbapenems: past, present, and future. Antimicrobial agents and chemotherapy. 2011;55(11):4943-60. https://doi.org/10.1128/AAC.00296-11

12. Haaber J, Penadés JR, Ingmer H. Transfer of antibiotic resistance in Staphylococcus aureus. Trends in microbiology. 2017;25(11):893-905.

https://doi.org/10.1016/j.tim.2017.05.011
13. Bitrus AA, Zunita Z, Bejo SK, Othman S, Nadzir NAA. In vitro transfer of methicillin resistance determinants mecA from methicillin resistant Staphylococcus aureus (MRSA) to methicillin susceptible Staphylococcus aureus (MSSA). BMC microbiology. 2017;17(1):1-10. https://doi.org/10.1186/s12866-017-0994-6

14. Laxminarayan R, Matsoso P, Pant S, Brower C, Røttingen J-A, Klugman K, Davies S. Access to effective antimicrobials: a worldwide challenge. The Lancet. 2016;387(10014):168-75. https://doi.org/10.1016/S0140-6736(15)00474-2
15. Mamun MM, Sorinolu AJ, Munir M, Vejerano EP. Nanoantibiotics: functions and properties at the nanoscale to combat antibiotic resistance. Frontiers in chemistry. 2021;9:687660. https://doi.org/10.3389/fchem.2021.687660
16. Laws M, Shaaban A, Rahman KM. Antibiotic resistance breakers: current approaches and future directions. FEMS Microbiology Reviews. 2019;43(5):490-516. https://doi.org/10.1093/femsre/fuz014
17. Soares S, Sousa J, Pais A, Vitorino C. Nanomedicine: principles, properties, and regulatory issues. Frontiers in chemistry. 2018;6:360. https://doi.org/10.3389/fchem.2018.00360
18. Jijie R, Barras A, Teodorescu F, Boukherroub R, Szunerits S. Advancements on the molecular design of nanoantibiotics: current level of development and future challenges. Molecular Systems Design & Engineering. 2017;2(4):349-69. https://doi.org/10.1039/C7ME00048K
19. Duan X, Huang Y, Agarwal R, Lieber CM. Single-nanowire electrically driven lasers. Nature. 2003;421(6920):241-5. https://doi.org/10.1038/nature01353

20. Pagar K, Ghotekar S, Pagar T, Nikam A, Pansambal S, Oza R, Sanap D, Dabhane H. Antifungal activity of biosynthesized CuO nanoparticles using leaves extract of Moringa oleifera and their structural characterizations. Asian Journal of Nanosciences and Materials. 2020;3(1):15-23. Available from: https://www.researchgate.net/publication/335790648
21. Chowdhury R, Khan A, Rashid MH. Green synthesis of CuO nanoparticles using Lantana camara flower extract and their potential catalytic activity towards the aza-Michael reaction. RSC advances. 2020;10(24):14374-85. https://doi.org/10.1039/D0RA01479F
22. Muzammil S, Hayat S, Fakhar-E-Alam M, Aslam B, Siddique MH, Nisar MA, Saqalein M, Atif M, Sarwar A, Khurshid A. Nanoantibiotics: Future nanotechnologies to combat antibiotic resistance. Frontiers in Bioscience-Elite. 2018;10(2):352-74. https://doi.org/10.2741/E827
23. Rehana D, Mahendiran D, Kumar RS, Rahiman AK. Evaluation of antioxidant and anticancer activity of copper oxide nanoparticles synthesized using medicinally important plant extracts. Biomedicine & Pharmacotherapy. 2017;89:1067-77. https://doi.org/10.1016/j.biopha.2017.02.101
24. Hajipour MJ, Fromm KM, Ashkarran AA, de Aberasturi DJ, de Larramendi IR, Rojo T, Serpooshan V, Parak WJ, Mahmoudi M. Antibacterial properties of nanoparticles. Trends in biotechnology. 2012;30(10):499-511. https://doi.org/10.1016/j.tibtech.2012.06.004
25. Mahapatra O, Bhagat M, Gopalakrishnan C, Arunachalam KD. Ultrafine dispersed CuO nanoparticles and their antibacterial activity. Journal of Experimental Nanoscience. 2008;3(3):185-93. https://doi.org/10.1080/17458080802395460
26. Wayne P. Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing. 2011. Available from: https://www.sid.ir/paper/655659/en
27. Tang J, Kang M, Chen H, Shi X, Zhou R, Chen J, Du Y. The staphylococcal nuclease prevents biofilm formation in Staphylococcus aureus and other biofilm-forming bacteria. Science China Life Sciences. 2011;54(9):863-9. https://doi.org/10.1007/s11427-011-4195-5

28. Lokhande PE, Deokar SV, Kulkarni PD, editors. Synthesis and Characterization of Nanostructured CuO Material for Tribological Application. Proceedings of TRIBOINDIA-2018 An International Conference on Tribology; 2018. https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3313502
29. Wan H, Li D, Dai Y, Hu Y, Liu B, Dong L. Catalytic behaviors of CuO supported on Mn2O3 modified γ-Al2O3 for NO reduction by CO. Journal of Molecular Catalysis A: Chemical. 2010;332(1-2):32-44. https://doi.org/10.1016/j.molcata.2010.08.016
30. Widiarti N, Sae J, Wahyuni S, editors. Synthesis CuO-ZnO nanocomposite and its application as an antibacterial agent. IOP Conference Series: Materials Science and Engineering; 2017: IOP Publishing. https://iopscience.iop.org/article/10.1088/1757-899X/172/1/012036/meta.
31. Kang J, Liu H, Zheng Y-M, Qu J, Chen JP. Systematic study of synergistic and antagonistic effects on adsorption of tetracycline and copper onto a chitosan. Journal of Colloid and Interface Science. 2010;344(1):117-25. https://doi.org/10.1016/j.jcis.2009.11.049
32. Macha IJ, Muna MM, Magere JL. In vitro study and characterization of cotton fabric PLA composite as a slow antibiotic delivery device for biomedical applications. Journal of Drug Delivery Science and Technology. 2018;43:172-7. https://doi.org/10.1016/j.jddst.2017.10.005