ABSTRACT

Bacterial resistance to antibiotics has become the greatest challenge, which has resulted in the search for efficient antibacterial agents. Metal oxides have exhibited significant antibacterial activity, which has resulted in apoptosis of bacterial strains. In this work, pure metal oxide nanoparticles, i.e., CuO and NiO along with mixed metal oxide nanoparticles i.e., CuO-NiO, NiO-ZnO and CuO-NiO-ZnO were synthesized and were also characterized using X-ray powder Diffraction (XRD) analysis, Field Emission Scanning Electron Microscope (FESEM) analysis, Fourier Transform-Infrared Spectroscopy (FTIR) and UV-Visible Absorption Spectroscopy. This work aims at studying the preliminary antibacterial activity of pure and mixed metal oxide (MMO) nanoparticles. The toxicity of the nanoparticle was assessed using Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli)*. Bacterial survival rate study, FESEM analysis and cell integrity study were carried out as the preliminary assessment of antibacterial activity of the MMO nanoparticles.

CuO-NiO showed enhanced antibacterial activity compared to CuO and NiO nanoparticles. In the second section, CuO, NiO and CuO-NiO nanoparticles' mechanism of interaction with *S. aureus* and *E. coli* cells was investigated. Change in the cell membrane's permeability and integrity was analyzed by the uptake of crystal violet dye in the outer bacterial membrane, release of β-galactosidase enzyme, and relative electric conductivity assay. Nanoparticles triggered the intracellular ROS generation in terms of superoxide ions, which caused severe membrane damage. Membrane destabilization was confirmed by zeta potential analysis, which led to surface charge neutralization. The change in the chemical composition of the cell surface, including proteins, carbohydrates, and fatty acids, was understood by FTIR analysis. The in-depth alteration of surface properties was given by AFM imaging analysis. The fragmentation of DNA and nanoparticle-cell attachment was confirmed by confocal microscopy analysis. A comparative study of these preliminary assessments was carried out between the pure metal oxide (i.e., CuO and NiO) and MMO CuO-NiO nanoparticles in order to understand the synergistic interaction of copper and nickel metals. This study showed that MMO nanoparticles could be used in the health and biomedical industry because of the enhanced antibacterial activity compared to a monometallic nanoparticle.

Keywords: Nanoparticle-cell interaction, Antibacterial activity, Mixed metal oxide nanoparticles