Potential Biomedical Applications of Zinc Oxide Nanoparticles (ZnO NPs) Obtained using Aqueous Extract of Green Algae *Cladophora* sp.

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Abstract

Algae encompass both prokaryotic and eukaryotic organisms that can develop in various ecological environments. They are notable for their high carbon dioxide sequestration rates, ability to accumulate toxic metals, low energy requirements, and as one of the richest sources of nutrients. Biomolecules present in algae, such as enzymes, pigments, flavonoids, and alkaloids, are highly important for the reduction and synthesis of metal and metal oxide nanoparticles. Therefore, nanoparticles produced via algae are easy and rapid to synthesize and do not contain any toxins. Among nanoparticles developed for medical applications, ZnO NPs exhibit low toxicity, are biocompatible, and cost-effective, making them valuable for studies in drug delivery, antimicrobial therapy, and cancer treatment. Nevertheless, concerns remain regarding their potential toxicity and environmental impacts. Further research is needed to ensure the stability of ZnO NPs and to minimize possible risks. The aim of this study is to investigate the potential biomedical applications of ZnO NPs that we previously synthesized and characterized using the aqueous extract of *Cladophora* sp. green algae. For this purpose, the cytotoxic effects of ZnO NPs on the healthy mouse fibroblast L929 cell line were evaluated using the WST-8 assay. Ten different concentrations of ZnO NPs (ranging from 0.95 to 500 µg/mL) were tested in the study. The IC_{50} dose was determined to be 7.73 µg/mL, and at this effective dose, apoptosis-necrosis and oxidative stress were examined using the MUSE Annexin V/Dead Cell assay. Results from the MUSE ROS kit showed that 81.49% of the cell population experienced oxidative stress following ZnO NP treatment. In the apoptosis assays, the decrease in cell viability from 85.35% to 41.90% indicates that ZnO nanoparticles exert a cytotoxic effect on L929 fibroblast cells. Early apoptosis decreased from 8.5% to 0.15%, while late apoptosis increased from 5.85% to 17.95%. These findings demonstrate that ZnO nanoparticles induce significant cytotoxicity in fibroblast cells, with cell death occurring predominantly in the late apoptotic phase and early apoptosis rapidly progressing to the late stage. Our data suggest that the use of ZnO NPs at low concentrations below the IC_{50} dose may have potential applications in various biomedical fields; however, further detailed studies are required to confirm these findings.

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