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ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN: 0976-3376

Asian Journal of Science and Technology
Vol. 17, Issue, 04, pp. 14228-14230, April, 2026

RESEARCH ARTICLE

BIOGENICALLY SYNTHESIZED GLUCOSE-CAPPED ZNO NANOPARTICLES AS SELECTIVE ANTICANCER AGENTS AGAINST TRIPLE-NEGATIVE BREAST CANCER

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ARTICLE INFO

Article History:
Received 16th January, 2026
Received in revised form
21st February, 2026
Accepted 28th March, 2026
Published online 30th April, 2026

Key words:
Anticancer, Biocompatible, Capping, Cytotoxicity, Glucose, Nanotherapeutics, Precision Oncology, Solanum nigrum, Surface Passivation, ZnO nanoparticles.

ABSTRACT

The selective targeting ability of nanoparticles has overshadowed conventional cancer therapies. Recent studies have reported the anticancer potential of ZnO nanoparticles due to their high biocompatibility and selectivity. Through this study, the anticancer potential of biogenically synthesized glucose-capped (GC ZnO-NPs) and uncapped ZnO nanoparticles (UC ZnO-NPs) by aqueous leaf extract of *Solanum nigrum* has been assessed against the triple-negative breast cancer (MDA-MB-231) cell line through induction of apoptosis and the generation of reactive oxygen species (ROS). The GC ZnO-NPs nanoparticles exhibited controlled cytotoxicity (IC_{50}=63.6 µg/mL) compared to UC ZnO-NPs(IC_{50}=108.8 µg/mL), which may be due to surface passivation that might have modulated nanoparticle-cell surface interactions. These findings suggest that modifying the surface properties of ZnO nanoparticles with biocompatible moieties can enhance their therapeutic potential, offering promising avenues for personalized nanotherapeutics and precision oncology.

Citation: ***Avni Nayyar, Shruti Anand, Afsheen Fatima, Manaal Zahera and Archna Talwar.*** **2026.** "Biogenically Synthesized Glucose-capped zno Nanoparticles as Selective Anticancer Agents against Triple-Negative Breast Cancer", *Asian Journal of Science and Technology*, 17, (04), 14228-14230.

INTRODUCTION

The traditional therapeutic modes of treatment of cancer are associated with adverse effects and are widely replaced by targeted drug delivery systems and nanomedicines (Pavlopoulou *et al*., 2016; Sevastre *et al*., 2022). The advanced therapeutic potential of nanomedicines enables selective targeting of nanospecies, offering key benefits such as active and passive targeting, enhanced bioavailability, and biocompatibility (McNeil, 2009). ZnO nanoparticles have been widely studied for their anticancer properties primarily through the generation of reactive oxygen species (ROS) and disruption of zinc-dependent protein homeostasis(Shen *et al*., 2013). It has been reported that phytofabricated ZnO nanoparticles show a dose-dependent increase in cytotoxicity against various cancer cell lines (Abbasi *et al*., 2019). Therefore, this study aims to investigate the anticancer potential of glucose-capped and uncapped ZnO nanoparticles against triple-negative breast cancer cells (MDA-MB-231). Recent investigations have demonstrated that the high surface reactivity of uncapped ZnO nanoparticles leads to rapid oxidative stress, ultimately resulting in mitochondrial dysfunction and cell death (Guan *et al*., 2012). However, capping of glucose reduces the agglomeration of nanoparticles through hydrogen bonding and exhibits higher cell viability than their uncapped counterparts(Sanyasi *et al*., 2016). Although synthesis and characterization of these biogenically synthesized nanoparticles from aqueous leaf extract of *Solanum nigrum* have been reported earlier in our research article (Talwar *et al*., 2025). The novelty of this report lies in its exploration of the cytotoxic and ROS-inducing effects of these compounds for the first time. This work demonstrates that surface functionalization with a biocompatible molecule (glucose) can mitigate nanoparticle toxicity, leading to more efficacious nanotherapeutics.

MATERIAL AND METHODS

Biogenic Synthesis of ZnO nanoparticles: The glucose-capped and uncapped ZnO nanoparticles were synthesized as described in ref (*Talwar et al*., 2025).

In vitro cytotoxicity assay of ZnO nanoparticles

The anticancer activity of biogenically synthesized ZnO nanoparticles was determined using an *in vitro* MTT assay against triple-negative breast cancer (MDA-MB-231) cell lines and normal cell lines, obtained from NCCS, Pune, India. The cells were seeded in 96-well plates at a cell density of 1×10^4 cells per well using DMEM-F12 media. The 96-well plates were incubated in 5% CO_2 atmosphere at 37°C for 24 hours. Subsequently, the cell lines were treated with different concentrations of ZnO nanoparticles in triplicate and incubated for an additional 48 hours. After 48 hours, the media from the cells was removed, and 20 µL of MTT reagent (5 mg/mL) was added to each well and incubated for 2-4 hours, until purple formazan crystals were formed. A 100 µL DMSO solution was added after removing the MTT reagent. The absorbance was measured at 570 nm using a microplate reader (BIORAD-PW41, USA).

ROS generation assay

Intracellular reactive oxygen species (ROS) generation in MDA-MB-231 cells was evaluated using the Dichloro-dihydro-fluorescein

diacetate (DCFH-DA) staining method. The cells treated with ZnO nanoparticles were exposed to 10 µM DCFH-DA dye to detect ROS generation. The fluorescent compound DCF produced by intracellular ROS is directly proportional to the level of ROS inside the cells. The readings were measured using a multiwell plate reader (BioTek's Synergy H1 Hybrid Multi-Mode Microplate Reader) at 485/528 nm of excitation/emission wavelength.

Statistical Analysis

The experiments were performed in triplicate, and the statistical analyses were conducted using Microsoft Excel to ensure the reliability of the results.

RESULTS AND DISCUSSIONS

In vitro cytotoxicity assay of ZnO nanoparticles

The *in vitro* cytotoxic effect of ZnO nanoparticles against MDA-MB-231 cells was investigated, and it was found that ZnO nanoparticles were statistically significant compared to that of the control (100% cell proliferation) in MDA-MB-231 at concentrations of60, 80, and 100 µg/mL (Figure 1). The synthesized ZnO nanoparticles were toxic to MDA-MB-231 cells at concentrations of 60 µg/mL and higher.

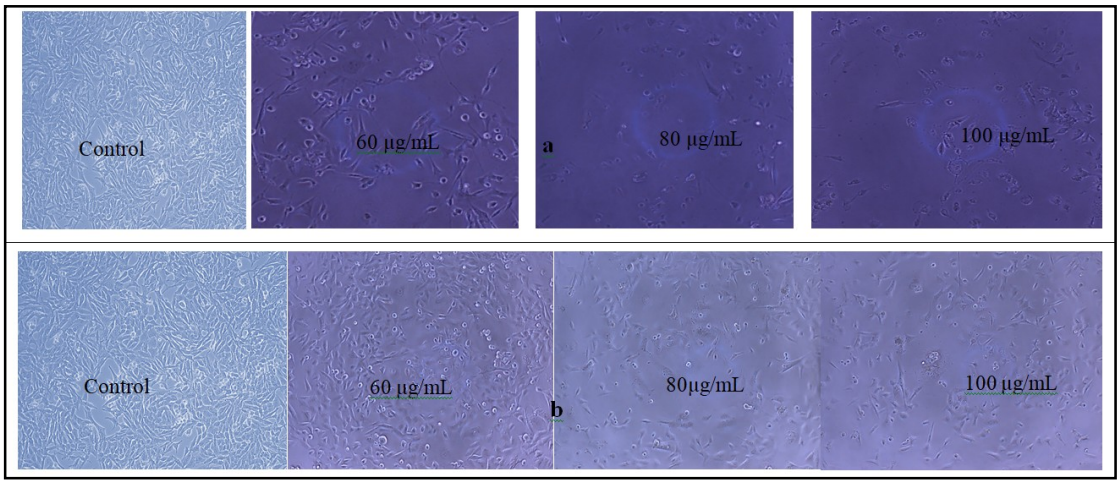

Figure 1. Microscopic images of cells treated with different concentrations of ZnO nanoparticles. (a) GC ZnO-NPs (b) UC ZnO-NPs

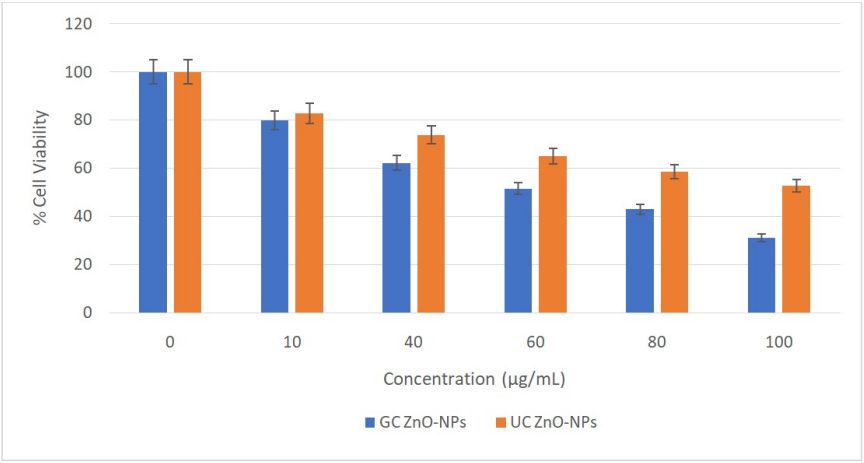

Figure 2. Dose-response effect of ZnO nanoparticles at different concentrations

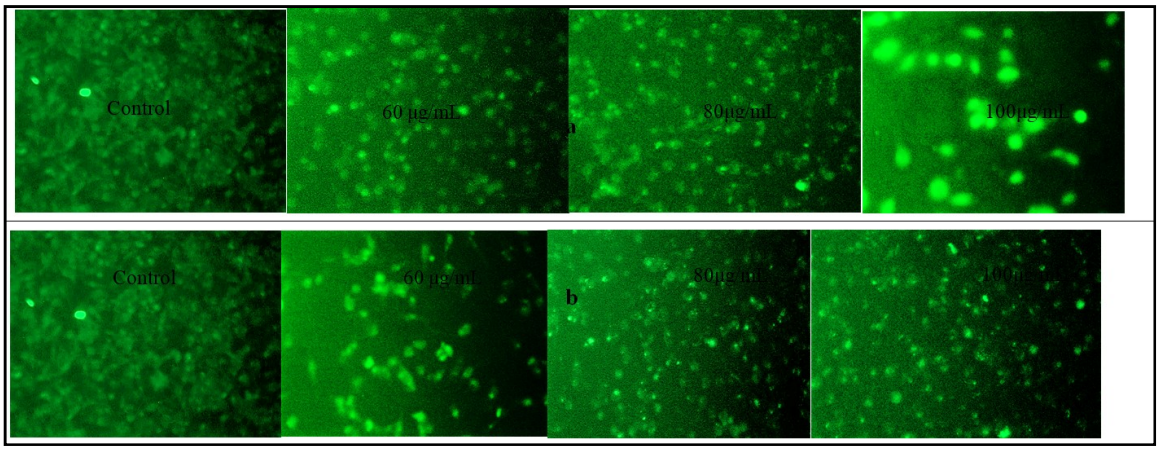

Figure 3. Photomicrographs showing intracellular ROS production at different concentrations of ZnO nanoparticles (a) GC ZnO-NPs(b) UC-ZnO-NPs

The calculated IC_{50} values for GC ZnO-NPs and UC ZnO-NPs were 63.6 µg/mL and 108.8 µg/mL, respectively, reflecting the higher cytotoxic potential of the glucose-capped ZnO nanoparticles (Figure 2).

ROS generation assay

The MDA-MB-231 cells were exposed to different concentrations of ZnO nanoparticles to determine the extent of ROS generation. Figure 3 illustrates a considerable increase in the level of intracellular ROS generation in GC ZnO-NPs and UC ZnO-NPs with increasing dosages of ZnO nanoparticles. However, images show that GC ZnO-NPs generate ROS in a controlled manner, thereby highlighting the role of the capping agent in enhancing the therapeutic selectivity of ZnO nanoparticles.

CONCLUSION

This study investigated the anticancer potential of glucose-capped and uncapped zinc oxide nanoparticles against triple-negative breast cancer cells (MDA-MB-231) by evaluating their therapeutic efficacy and biocompatibility profiles. The results demonstrated that glucose-capped ZnO nanoparticles are significantly more biocompatible than uncapped ZnO nanoparticles. It can be proposed that glucose coating can modify the physicochemical properties of nanoparticles, reduce unfavorable electrostatic interactions with cellular membranes, and minimize collateral damage to normal cells. This differential uptake of monosaccharide-coated ZnO nanoparticles could enhance their recognition of cancer cells and improve their therapeutic effectiveness. However, further investigations are required to evaluate the *in vivo* efficacy of these nanoparticles. Thus, these findings suggest that surface functionalization of nanoparticles with a biocompatible moiety can minimize their adverse effects and may offer avenues for the advancement of personalized and precision oncology.

Acknowledgments

The authors are grateful to Prof. Abbas Ali Mahdi, Vice Chancellor, and Pro-Vice Chancellor Prof. Farzana Mahedi, Era University, Lucknow, India, for providing analytical and laboratory facilities.

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