# Green Synthesis and Cytotoxicity of CdO/Biochar Nanocomposite Against MCF-7 Cells

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Abstract: The growing trend in breast cancers worldwide proves the significance of the development of tough nanoparticle materials with high therapeutic potential. We demonstrate a straightforward green precipitation technique for synthesizing cadmium oxide nanoparticles utilizing cadmium nitrate as a precursor and olive leaf extract as an environmentally benign capping and stabilizing agent. Additionally, biochar obtained from household waste was utilized as a porous carbon scaffold to create a cadmium oxide/biochar binary nanocomposite, enhancing structural stability and preventing agglomeration. Characterization of Prepared Materials via X-ray diffraction, Field emission scanning microscopy, Energy-dispersive X-ray spectroscopy. The cytotoxicity (D) of pure cadmium oxide nanoparticles and (B) the cadmium oxide/biochar nanocomposite against the human breast cancer cell line (Michigan Cancer Foundation-7) as determined by MTT assay. The cadmium oxide/biochar nanocomposite (B) had the best anticancer effect, with the lowest $IC_{50}$ value (5.86 µg/mL), compared to pure cadmium oxide nanoparticles (D). The performance is improved because the biochar support, in combination with nanoparticles, enhances cellular uptake, leading to cancer cell death via Reactive oxygen species under near-infrared light. These findings collectively indicate that the cadmium oxide/biochar nanocomposite holds significant potential as a cost-effective and effective treatment platform for breast cancer.

## 1 INTRODUCTION

Cancer is still among the most prominent unmet medical needs of our time and a leading cause of death globally, due to the complex biological characteristics that allow tumor cells to escape from systemic immune surveillance [1]. Breast cancer represents a significant socioeconomic burden of global concern, being the most common malignancy among women [2]. The primary therapeutic challenge presented in breast cancer therapy is the development of multidrug resistance to conventional chemotherapies that are non-specific and induce severe systemic toxicity by injuring normal tissues [3]. Nanomaterials have been used to overcome these drawbacks by offering specific distribution and increased cellular uptake via the EPR effect. To achieve this, they cause tumor cells to produce Reactive Oxygen Species (ROS), which cause oxidative stress and programmed cell death (apoptosis) with minimal destruction of normal tissues [4]. Certain recent reports have demonstrated that this strategy works; such as Lefojane et al., demonstrated that CdO has a high cytotoxicity effect on breast cancer, and Abu Hajleh et al., have demonstrated that biochar scaffolds do not allow nanoparticles to clump together, a process that significantly increases their biological activity [5], [6]. To enable targeted treatments to be developed, the Michigan Cancer Foundation-7 (MCF-7) human breast adenocarcinoma cell line is a widely used gold-standard laboratory model, as it mimics estrogen-receptor-positive breast tumor molecular responses [7]. Biogenic nanotechnology is thus emerging as a promising strategy, providing an environmentally friendly and sustainable approach for cancer treatment with improved biocompatibility/dispersity [8]. Metal oxide nanoparticles, especially cadmium oxide (CdO) have received great interest in nanomedicine because of their unique physicochemical properties and an electronic bandgap that may enable them to produce effective biological interactions with tumor cells [9]. We have also recently reported that the nanoparticle form, CdO shows reduced systemic toxicity compared to other metallic semiconductors, which

indicates a promising application for local cytotoxicity targeting of tumor cells under an appropriate dosage with no effect on normal mammalian cells [10]. Nonetheless, a key technical challenge of bare CdO nanoparticles is their relatively higher surface energy, which can cause agglomeration and significantly decrease the effective area of the active molecules charged in CdO for bio-therapy [11]. In order to stem this, the development of hybrid nanocomposites with structural supports has become crucial [12]. The biochar is a highly porous carbonaceous material obtained through biomass conversion which can be used as the ideal structural support due to its high surface area and the functional groups, thus facilitating the homogeneous distribution of CdO NPs by preventing agglomeration and allowing for cellular membrane penetration of the tumor. Such hybrids are typically synthesized via advanced methods such as co-precipitation, which facilitates tunable design for the particle architecture [13]. The resulting CdO/Biochar nanocomposite operates by a synergistic mode of action that results in the overproduction of the Reactive Oxygen Species (ROS) inside the cancer cells, ensuing catastrophic oxidative stress and the consequent DNA damage [14]. Further optimization of such systems towards biomedical applications was achieved by increasing cellular uptake (e.g., attachment of carbon quantum dots, CQDs) or higher imaging capabilities [15]. The use of biowaste for the production such carriers not only reduce overall toxicity but also is in accordance with the principles defined by green chemistry [16]. MCF-7 cells exhibit a strictly dose- and size-dependent cytotoxic response to these nanoparticles [17], which have been shown to activate a mitochondrial pathway of apoptosis (programmed cell death) [18]. In addition, the "green synthesis" of the nanoparticles with the plant extracts enables their capping with bioactive molecules that promote stability and biological affinity of nanoparticles [19], thereby enabling further development towards advanced theranostic platforms [20]. The pro-oxidant activity against tumors requires the electron transfer processes facilitated by biochar, which also provides a protective scaffold to avoid premature degradation of the active phase [21], [22]. They stand as the most effective inhibitors of vital metabolic control points like (malate dehydrogenase; MDH-1), which is essential for the well-being of cancerous cells [23]. Advanced characterization tools like XRD and FE-SEM are being used to correlate the structural morphology of these systems with their biological performance. [24] Whether cadmium-based or hybrids of selenium, these new delivery systems have revolutionized the advances towards intelligent drug delivery against molecular abnormalities associated with breast cancer [25]. The hypothesis of the study is that the stability and dispersion of green-synthesized CdO nanoparticles as a support with the help of a biochar matrix will be enhanced because the nanoparticles will not agglomerate on the surface of the matrix. It is anticipated that this synergistic mixture will enhance the active surface area and ease the uptake by the cell, and hence, boost the cytotoxic impact on the MCF-7 breast cancer cell line as opposed to the unsupported nanoparticles.

# 2 EXPERIMENTAL

## 2.1 Materials and Reagents

The chemicals used in this study were obtained from commercial suppliers and are commercially available in analytical-standard quality; no further purification was performed. This was accomplished using cadmium nitrate tetrahydrate, $Cd(NO_3)_2 \cdot 4H_2O$, as the principal precursor for cadmium oxide nanoparticles, procured from 99% purity, Alpha Chemical (India), with a molecular weight of 308.48 g/mol and purity of 99%. For the adjustment of pH in the reaction mixture, sodium hydroxide (NaOH, 98% pure) from SDS Fine Chemicals (India), was used as a precipitating agent. Subsequent washing and purification steps used absolute ethanol (99.9%) from BDH Chemicals (England) and deionized water. To obtain CdO nanoparticles, an environmentally friendly process was used, which presupposed the use of an aqueous plant extract prepared of the locally obtained olive leaves (Olea europaea). Additionally, biomass residues (household waste) were also collected locally for being processed as feedstock to produce biochar.

## 2.2 Instruments

Different laboratory and analytical tools were used. Digital magnetic stirrer (MS-H280-pro, GMBH, Germany) and programmable muffle furnace (LEF-105S-1, Lab Tech, Korea) were used in the Department of Chemistry, College of Education, Pure Sciences, Diyala University, Iraq. The pH measurements were done on a calibrated pH meter (Seven Compact, Mettler Toledo, Germany). A high-energy planetary ball mill (E-Max, Retsch, Germany) was used to prepare the crushed biochar. An

ultrasonic bath (DK Sonik, China) was used for the sample's homogeneous dispersion. A crystalline structure was characterized by powder X-ray diffraction (XRD, Bosch D500 diffractometer, Germany). The surface morphology and elemental composition were investigated using a FE-SEM, EDS (Germany).

## 2.3 Preparation of Olive Leaf Extract

In order to maintain the purity of the olive leaves (Olea europaea) by eliminating environmental pollutants, dust, and grime, the leaves were washed several times using deionized water upon being picked from the olive tree. When the leaves were cut, they were stored away to dry in the sunshine for at least five days. Individual extraction would be made by drying the materials and grinding them into fine powder. The aqueous extract was then prepared by adding 20 g of olive leaf powder to 200 mL of deionized water in a glass flask. To allow for efficient extraction of phytochemicals, the mixture was shaken on a hot plate at 70 °C for 30 min using continuous magnetic stirring. Olive leaf extract (OLE) can serve two main roles: it can play the role of a natural reducing and capping agent for the nanoparticles, but more importantly, it provides biogenic protective layer to minimize inherent toxicity of cadmium. The filtered extract has removed the soluble pollutants from the olive leaf extract, leaving a clear OLE.

## 2.4 Preparation of Biochar

Biochar was produced from local residues, more specifically obtained from household waste. Then the biomass was dried and thermally decomposed (pyrolysis) at controlled conditions in a muffle furnace. Carbonization was performed for 5 h in oxygen deficient environment at 600 °C. The biochar mass thus produced, which was carbon-enriched by weight, was cooled and ground into a fine powder by a planetary ball mill with rotation speed 400 rpm for the time of 1 h.

## 2.5 Synthesis of Cadmium Oxide Nanoparticles

The cadmium oxide nanoparticles were synthesized by using a plant-based chemical precipitation method with olive leaf extract (OLE) as a natural stabilizer and capping agent. The pre-cursor metal solutions were prepared by dissolving 2 g of cadmium nitrate in 200 mL of deionized water. Then, 10 mL of OLE was gradually added through a burette dropwise; a visible color change at this moment indicated the successful chemical interaction between phytochemicals in the extract and cadmium ions. Cohesive formation of the hydroxide intermediate was achieved by slow addition of a 1 M NaOH solution (2 g dissolved in 50 mL distilled water) until pH 11–13, monitored on a calibrated pH meter. The precipitate was recovered by vacuum filtration, washed with four portions of ethanol to remove organic matter and then with deionized water until a neutral pH (7) was reached. The purified solid was dried at 100 °C for 2 h, ground to fine powder, and calcined in a muffle furnace at 500 °C for 5 h to obtain crystalline CdO nanoparticles. Cadmium hydroxide first formed in the precipitation step and thermally decomposed during calcination in accordance with the following reaction [26]:

$$Cd(OH)_2 \rightarrow CdO + H_2O.$$

## 2.6 Synthesis of CdO/Biochar Binary Nanocomposite

Binary nanocomposites were generated using a solution-processing method for thermal stabilization. Preparation of CdO/biochar nanocomposite involves the dispersion of 0.5 g CdO nanoparticles and 1 g biochar (mass ratio 1:2) in 20 mL ethanol, then placed in ultrasonic bath and sonicated for 30 min at 50 °C to homogenize dispersion. The CdO nanoparticle suspension was mixed with the biochar suspension and again, the combined mixture was sonicated for another 3 hours at constant temperature of 55 °C for effective immobilization and attachment of nanoparticles on porous biochar surface. The composite was filtered, dried at 50 °C for two hours before a third calcination step of 600 °C. The intentional anchoring of the formed CdO nanoparticles onto the biochar matrix and the biogenic layer added to navigate capping with OLE safety-by-design approach. Such a structural design can control the release profile of cadmium ions, minimize possible non-specific interactions and limiting cytotoxic effects to being limited only in biological tests.

## 2.7 Characterization Techniques

Different analytical methods were utilized to investigate the structural, chemical and morphologic characteristics of synthesized CdO nanoparticles and CdO/biochar nanocomposite. X-ray diffraction (XRD) was used to determine the crystalline nature and phase purity of the synthesized materials. Surface

morphology and particles Distribution of the nanocomposite were examined by Field Emission Scanning Electron Microscopy (FE-SEM) and to investigation the elemental composition of the nanocomposite energy-dispersive X-ray spectroscopy (EDS/EDX) was employed.

## 2.8 Cell Culture and Cytotoxicity Evaluation (MTT Assay)

Cytotoxicity of the synthesized compounds was evaluated against a human breast cancer cell line (MCF-7) from the Pasteur Institute of Tehran, Iran. All the experiments were performed at the RASA Research Laboratory in Tehran, Iran. MCF-7 cells were grown in RPMI-1640 (Sigma-Aldrich) containing all essential nutrients in a humidified incubator at 37 °C and 5% $CO_2$. The cultures were examined daily with inverted microscopes to ensure normal growth and to confirm the absence of microbial contamination. To make a stock solution for the cytotoxicity assay, either nanomaterial dissolved in DMSO (5 mg/50 µL). The compounds were diluted with culture medium to prepare final test concentrations in the solutions. Preparation of MTT Reagent: We prepared 5 mg/mL of tetrazolium powder finally dissolved in Phosphate Buffered Saline (PBS) The mixture was then vortexed for a short period of time, filtered through a 0.2 µm syringe filter, and stored at 20 °C in opaque amber vials. In the experimental method, MCF-7 cells ($1 \times 10^4$ cells/well in 100 µL), after which they were left to adhere for 24 hours in 96-well microplates. We then added 100 µL of the already prepared nanoparticle solutions to each of the three wells. 100 µL of cell culture supernatant in the wells containing 10 µL of MTT solution were incubated for 24 hours and then gently shook to promote mixing. The plates were then incubated at 37 °C for 5 h, allowing live cells to generate purple formazan crystals via metabolic reduction of the dye. At the termination of the incubation period, the culture medium was gently removed from each well. Then, after the formazan crystals were formed, 100 µL of DMSO was added to each well of the micro-plates and incubated until a pink-purple liquid appeared, dissolving the crystals. The absorbance, or optical density, was read at 570 nm with a Dana 3200 kit microplate reader (Iran, 2006) T X-ray diffraction. To obtain the percentage of viable cells using the following formula, we compared treated and untreated cells [27]:

$$\text{Cell viability (\%)} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \times 100.$$

Absorbance of treated cells ($A_{treated}$) and absorbance of untreated control cells ($A_{control}$).

The $IC_{50}$ values (the concentration that results in 50% inhibition of growth).

# 3 RESULTS AND DISCUSSION

## 3.1 XRD Analysis

X-ray diffraction (XRD) was used to characterize the crystalline structure, phase purity, and the successful loading of the materials that were synthesized. The diffraction patterns of pure CdO NPs and CdO/Biochar nanocomposite are shown in Figure 1. The XRD pattern of the pure CdO NPs (Fig. 1a) shows a number of intense and sharp reflections at θ values of roughly 33.02°, 38.30°, 49.67°, 55.28°, 65.92° and 69.27°. The dominant peak found at θ = 33.02° evidence that the CdO nanostructure presents a preferential orientation. These characteristic peaks correspond very well to the standard cubic cadmium oxide (CdO) data (JCPDS card No 05-0640) indicating the high crystallinity of the synthesized material as the target compound. Minimal Additional Peaks: The absence of any additional peaks signifies that the proposed green synthesis procedure has been successful in inhibiting the formation of any secondary phases or impurities. For the CdO/Biochar nanocomposite Figure 1b, although the characteristic diffraction peaks of the cubic CdO phase are still present, their sharp and well-defined nature confirms that crystalline integrity of the nanoparticles were maintained after being dispersed onto the support. The nanocomposite possesses a structural advantage over the bare oxide. The faint broadening and finding a broad background feature between 20° and 30° are due to the amorphous carbon matrix of household waste biochar. Structurally, CdO/Biochar nanocomposite is more enhanced than the pure CdO NPs. The XRD results indicate that, due to the stability of the biochar as a physical support, a better dispersion of the particles occurs. Intrinsic to the system, pure nanoparticles tend to reside in clusters therefore their surface area becomes inactive due to agglomeration, and this can be less effective than the presence of biochar phase (as shown by softening the lattice strain coupled with the broadening of the amorphous part, Figure 1b confirming a synergistic system having formed. This structural modification is important for improving the interaction of nanoparticles with biological targets; hence the

composite is a stronger cytotoxic agent than the unsupported CdO NPs.

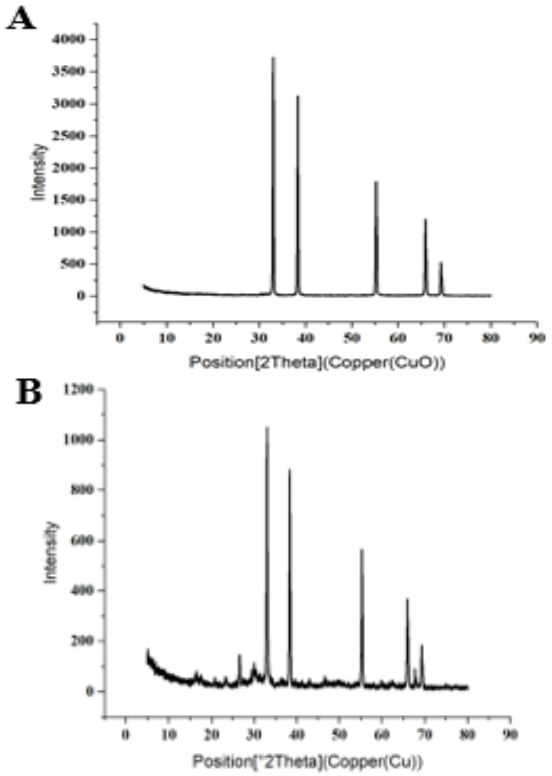


Figure 1: XRD patterns of: a) CdO nanoparticles green synthesized, b) CdO/Biochar nanocomposite.

## 2.9 FE-SEM Analysis

The surface morphology and particle size studied by FE-SEM for the synthesized materials is depicted in Figure 2. The FE-SEM micrograph for pure CdO NPs is presented in Figure 2a. The particles display a non-uniform, rod-like distribution with a strong propensity for self-aggregation into clumps. This process of agglomeration is a common feature of metal oxide nanomaterials, caused by high surface energy and robust inter-particle van der Waals forces. The direct measurements of these nanoparticles revealed size distribution between 20.73 nm to 77.2 nm, confirming that the nanostructures had been formed using the green synthesis method. The morphology of CdO/Biochar nanocomposite is shown in the Figure 2b. There is an important change in the structure; the CdO nanoparticles are clearly spread over the biochar support surface which is porous and layered. This biochar matrix, which derives from household waste, serves as a stabilizing scaffold and significantly prevents the large agglomeration observed in pure oxide sample. As compared to Figure 2a, the nanocomposite is clearly superior as shown in Figure 2b. The biochar acts as a physical barrier in the composite, preventing particle growth and agglomeration, thereby enhancing the uniform dispersion of active CdO sites. The particle sizes of the composite appear more controlled and better anchored, which is key to accessing all of the surface area. The formation of this dispersed, and aggregate-free system of CdO/Biochar should enhance biological interaction and hence cytotoxic potential over that of unsupported CdO NPs.

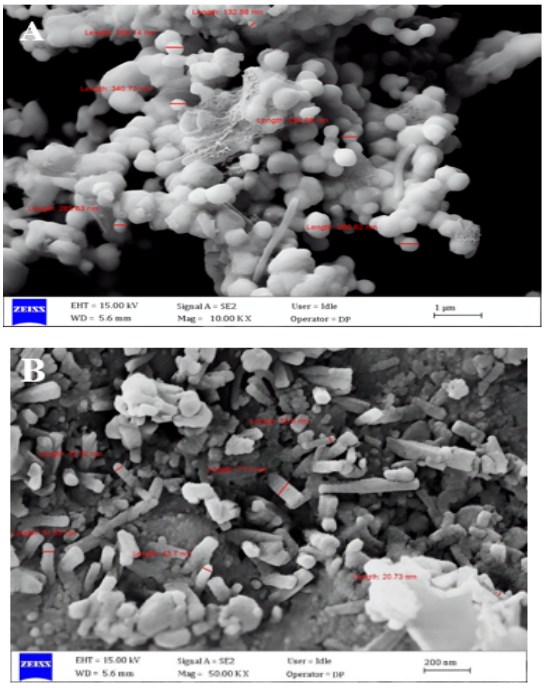


Figure 2: FE-SEM micrographs of: a) pure CdO nanoparticles showing agglomerated clusters, b) CdO/Biochar nanocomposite showing improved dispersion on the biochar support.

## 2.10 EDS Analysis

The composition and phase purity of the selected materials were systematically characterized by means of EDS attached to the FE-SEM. Figure 3 presents the energy dispersive X-ray spectroscopy (EDX) spectra as well as the corresponding semi-quantitative elemental statistics of the pure CdO NPs and the CdO/Biochar nanocomposite. As in Figure 3a, the generic features of Cadmium (Cd) and Oxygen (O) could be identified, which verified the oxide nanostructure, in terms of the pure CdO NPs. Based on quantitative data provided in the Spectrum 15, the major element is Cadmium (Cd) which has a wt of

87.6 wt and Oxygen (O) has a wt of 5.9 wt. There is also a signal at 5.6 wt% of the spectrum indicating Carbon (C) which is an experimental artefact probably due to the carbon tape that was used to mount the samples. Sulfur (S) was found in very low quantities (0.9 wt) and this could be explained by the presence of organic remains or remnants of precursors. CdO/Biochar nanocomposite Figure 3b, the analysis of Spectrum 14 shows that the carbon matrix was incorporated successfully. The content of Cadmium (Cd) is 52.1 wt. and the content of Oxygen (O) goes up to 27.6 wt. The level of Carbon (C) at 14.0 wt is also notably high, which reflects the anchoring of the nanoparticles on the biochar support. The spectrum was also used to identify Sulfur (S) at 4.6 wt% and Copper (Cu) at 1.7 wt% which is considered to be an artifact of the mounting holder or the coating process. The peak intensities of Cd and O of the two samples in the spectrum are very high, which indicates not only the successful synthesis of crystalline CdO but also its composite as indicated by the structural data of XRD.

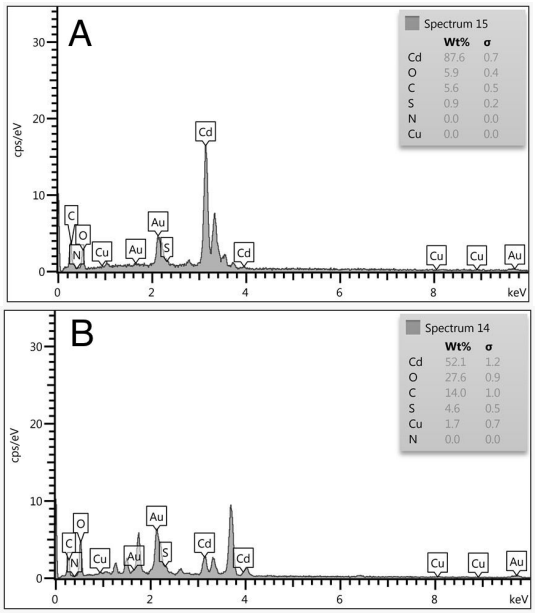


Figure 3: EDS Analysis of Synthesized Materials: a) The spectrum of Pure CdO NPs, b) The spectrum of CdO/Biochar nanocomposite.

## 2.11 Cytotoxic Activity of the Synthesized Nanocomposite Against MCF-7 Breast Cancer Cells

The toxicity of the prepared compounds to the human breast cancer cell line (MCF-7) using MTT assay showed the presence of a certain dose-dependent effect on pure CdO nanoparticles (D) as well as CdO/ biochar nanocomposite (B). Figure 4 shows that cell viability decreased with the rise in the concentrations of the treatment between 3.125 and 800 µg/mL. However, the CdO/ biochar nanocomposite (B) was capable of inhibiting cell growth with better capacity compared to the pure CdO nanoparticles (D). Such difference in biological activity could be attributed to the fact that pure nanoparticles (D) are apt to agglomeration due to the high surface energy that reduces their effective surface area and contact with the cells. On the other hand, in sample (B) where the biochar was encapsulated within porous biochar matrix, the CdO NPs were more stable system in which the biochar acted as a scaffold to allow the particles to be distributed uniformly and preventing clustering of the particles. This organization of the structure probably promoted cellular uptake and the following production of reactive oxygen species (ROS). These results are further indicated by the values of $IC_{50}$ of Figure 5; the nanocomposite (B) had a high inhibitory concentration of 5.86 ug/mL, which is much less than the inhibitory concentration of the pure CdO (D). These results indicate that nearly half of the concentration of the supported nanocomposite is required to inhibit 50% of growth of the cancer cell in comparison to the unsupported one suggesting that the biochar support is a good method of enhancing an anti-cancer effect of the CdO nanoparticles.

# 3 CONCLUSIONS

An integrated nanocomposite was prepared by synthesizing environmentally biosafe cadmium oxide (CdO) nanoparticles and integrating the formed nanoparticles with biochar derived from household waste. The biochar matrix ensured an effective porous support, thus preventing the aggregation and sintering of the CdO nanoparticles, as proved by structural and morphological studies. The biological outcomes showed that CdO/biochar nanocomposite (B) was more effective than the pure CdO NPs (D) against the MCF-7 cell line. The very low $IC_{50}$ value (5.86 µg/mL) suggests a synergistic effect of the biochar scaffold, enhancing both the reactivity and bioavailability of the nanoparticles. An important advantage of the study is a simple and inexpensive biotechnological approach to recycle household waste into biological substances, which may be used for breast cancer targeted therapy.

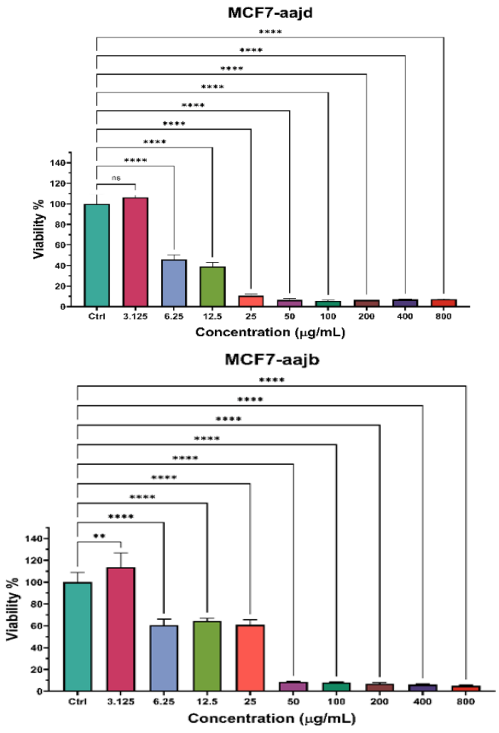


Figure 4: The cell viability (XTT assay) and morphological alterations of Michigan Cancer Foundation-7 breast cancer cells after being treated with (D) pure CdO NPs and (B) CdO/Biochar nanocomposite.

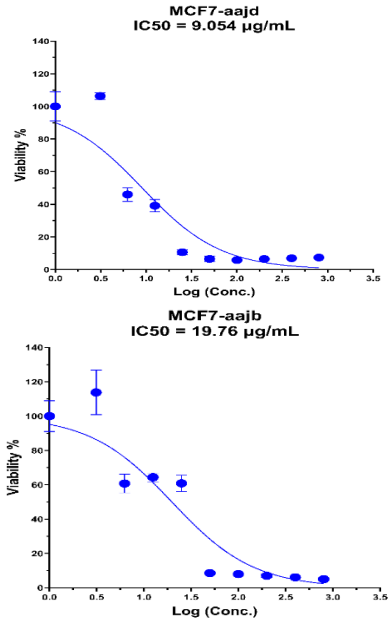


Figure 5: $IC_{50}$ values of pure CdO NPs (D) and CdO/Biochar nanocomposite (B) against MCF-7 cell line showed that the nanocomposite was effective at lower concentrations compared to pure CdO NPs suggesting that the nanocomposite was more potent.

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