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RESEARCH ARTICLE

Biosynthesized Co-ZnO Nanorods against breast cancer cells

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ABSTRACT

Through a successful attempt, the extract of *Prosopis fracta* was exerted for the green and simple production of pure and 3% cobalt doped zinc oxide Co-ZnO) nanorods (NRs), which were configured in the following through the analytical results of XRD, FESEM, and EDX procedures. The appearance of finely doped cobalt throughout the construction of zinc oxide was approved by the data of XRD and EDX. Considering how the length and diameter of pure ZnO Nanorods were determined by the FESEM process to be 500 ± 0.2 nm and 100 ± 5 nm, the doping process of cobalt into ZnO caused an enlargement respecting the doped nanorods length as well as diameter. We examined toxicity of nanorods towards breast cancer MCF-7 by the employment of WST-1 trial. In contrast to results of pure nanorods, the doped nanorods were abled to induce a stronger toxicity on MCF-7 cells and therefore, it can be indicated that the conduction of doping process on ZnO nanostructure resulted in intensifying its inhibitory impact towards MCF-7 cells.

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INTRODUCTION

The novel and interesting topic of green synthesizing procedures for metal nanoparticles, as well as the performance of their biosynthesis through organisms, has captured the focus of many scientists in nanoscience [1, 2]. Plants stand as the best choice for biosynthesizing nanoparticles and manufacturing products with higher stability and diversity in shape and size when compared to other ingredients [3, 4]. The application of chemical manufacturing approaches similar to micelles and hydrothermal, etc., results in the entry of toxic adsorbent chemical species to surfaces, which would consequently induce side effects in medical implementations. The interest of many has been invested in nanobiotechnology as the most promising field of nanoscience and nanotechnology in this era. Many aspects of human life can be literally and notably impacted by the fascination features of nanoparticles such as size, shape, and morphology [5-8]. In comparison to other similar products, the utilization of metal oxide nanoparticles [9].

As a novel product, researchers have noticed the broad utilization of zinc oxide nanoparticles among other mineral particles, which is the result of their physical and chemical qualities and their superior adsorption power in collation to the alternative zinc-holding compounds [10]. Zinc oxide succeeded in achieving the approval of US Department of Food and Drug Administration as a harmless compound [10]. There are proofs on the biocompatibility and non-toxicity of these products, as well as their applicability in the various form [11]. Concerning

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the notable qualities of Zinc oxide nanoparticles, one can mention their chemically powerful stable position, accommodation of low dielectric constant and superior photocatalytic functionality, ability to absorb infrared and ultraviolet light, and exhibition of particularly antibacterial and anti-cancer features [11-14].

With six critical physiognomies, carcinogenesis ensue in each cell, tissue, and organ, leading to the deteriorating variations that reason a great quantity of malignancies [1]. A complex process, carcinogenesis is primarily sparked by both genetic predispositions and environmental factors. Every year, there are alarmingly more fatalities from cancer, making it one of the most prevalent causes of mortality globally [3-5]. Even though a substantial number of malignancies aren't always fatal, they significantly decrease the standard life span and typically incur greater expenses. The handling of patients with BC is impacted by the biological subtypes' insights into cutting-edge therapy strategies and patient classifications. A novel rating system for BC is presented in the eighth version of the TNM categorization, which considers physiological variables in addition to physical traits [6]. Treatment of BC is complex and necessitates a number of methods, ranging from surgery to chemotherapy and etc [7].

The combination of drugs side impacts with time consuming, expensive, and inefficient treatments legitimatizes the rise of a worldwide urge for the composition of novel approaches and strategies to control this illness [17].

As a novel and promising knowledge, there are high expectations from the great strides of nanoparticles technology towards the manufacturing of numerous drugs, efficient treatments, and diagnosis routes for a large number of disorders that also involve cancer [18, 19]. There is a long history behind the consideration of metal oxide nanoparticles as an appropriate candidate for the treatment of cancer [20, 21]. These nanoparticles processing and synthesis has the potential of standing among the least expensive synthesizing protocols due to the availability of bountiful sources for organic metal oxides in nature. In this regard, they may prove to be efficient for this kind of cancer due to the qualities of zinc oxide nanoparticles [21].

Methylene blue, methylene red, and orange acid 7 are just a few examples of the highly concentrated dyes which the apparel sector releases into the surroundings. This is a significant problem because it can lead to serious contamination of the environment and make it easier for cancer-causing agents and contaminants [22]. These companies' effluent has high amounts of both organic and inorganic substances. The effluent from the textile and other sectors contains an important class of contaminants known as biological dyes [23]. Given a share 60 percent of all colours used in the apparel sector, Azo Group is by far the biggest and most crucial family of such pigments [24].

These facts pushed the aim of our study towards the exploitation of green synthesizing methods for pure and cobalt doped zinc oxide nanorods with cobalt through the exertion of *Prosopis fracta* extract. Contemplating the features of zinc oxide, the significance of cancer treatment in medicine, and the annihilation of environmental pollutants in industry, we attempted to assess the employment and impacts of synthesized NRs on the cytotoxicity of human breast cancer MCF7 cells.

MATERIALS AND METHOD

Arrangement of pure and Co-ZnO NRs

P. fracta extraction was obtained to start the specimen organisation process. Over this purpose, we weighed and added P. fracta bark powdered to distilled water in a 1:5 ratio while spinning it for 10 h at 200 rpm. Following the combination's filtering via the filters, the resulting substance was used in the experiments that followed. The process was then continued by adding 10 mL of extraction to two independent Erlenmeyer's that had been filled up to 70 mL with distilled water and put in a bath of water that was heated to 70 °C. Once the mixtures were stirred for 3 hours, the drying process was performed at 90 ˚C for the duration of 20 hours. As the last step, a furnace was utilized to calcine the dried samples at 600 ˚C for 1.30 hours.

Characterization of NRs

The characteristics of both pure and coated ZnO NRs were distinguished using a variety of testing techniques. The PXRD outcomes for yield were used to assess its crystalline their nature, and the Raman spectra were utilised for recording its vibration patterns. We also conducted FESEM analysis to evaluate the exterior shape of the specimens.

Cytotoxicity assessment

Cell culture

This section implicated the cytotoxic examination of NRs on breast cancer MCF7. For

this aim, the obtained breast cancer MCF7 cells from Pasteur Institute of Iran were defrosted in advance of the culturing process. Then, we conveyed the cells to Falcon tubes to begin their centrifugation by 833 rpm for the duration of ten min. The exertion of DMEM culture medium was considered to perform cell culturing next to inhibiting the microbial growth by adding 10% fetal bovine serum (FBS), 100 µg/mL of streptomycin, and 100 international units/mL of penicillin to every culture medium. The incubation of culture medium under 5% CO_2 at 37 °C as the next step facilitated the proliferation and growth of cells.

WST-1 Test

The execution of WST-1 examination helped in assessing the cytotoxic impacts of NRs in opposition to human breast cancer MCF7, while the five samples were evaluated in the sequent volumes of 1-1000 µg/mL as the role of control group was given to cell culture medium. To begin the process, a cell suspension composed of 10^5 cells/ well was appended to the wells of 96-well plate for undergoing an incubation procedure of 24 hours. The draining of culture medium out of every well of 96-wells plate was conducted. In the following, we put 80 µL of the product into the wells for incubating the plate throughout 1 day. Following dispatching, we appended 5 µL of WST-1 solution to every well for undergoing another 4 hours incubation. Lastly, an ELISA reader was exerted to determine the adsorption of every sample at the 480 nm. Next section implicated the configuration of cell viability (survival) percentage by applying the provided formula:

(%) = [100 × (sample abs)/ (control abs)].

IC50

The execution of a probit analysis necessitated the use of Graph pad prism to calculate the medication amount and the IC50 for NPs' ability to stop 50% of cell viability. This technique was also used to determine the percentage of the cell viability that was controlled by the used amount.

RESULTS AND DISCUSSION

XRD analysis

The XRD results of pure and 3% Co-ZnO NRs are shown in Fig 1. According to the PXRD examination of pure ZnO NRs, the hexagonal shape of the wurtzite structure of pure ZnO is indicated by the peak intensities [20]. When the rate of infused cobalt in the ZnO system was increased, the information displayed in Figure 1 showed a small change in the position of ZnO (101). This finding may be explained by the inconsistent substitute of Co ions all over the ZnO lattice. The particle size of the synthesised compounds was determined using Scherrer's equation in the sections that follow. The values were 18.65 and 29.8 nm for pure and 3% for Co-ZnO NRs, correspondingly. As a result, the NRs crystal size was increased by increasing the doping volumes of cobalt, that is likely related to the larger ionic radius of Co2+/3+, to be 0.75–0.9 cobalt as contrasted to the measure of zinc (0.74 Å).

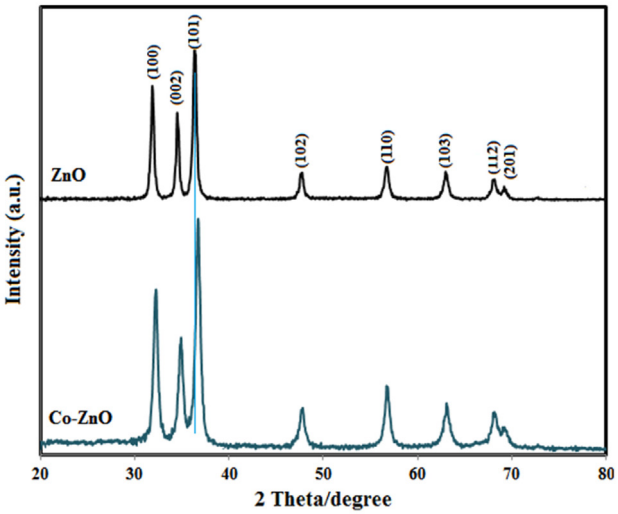

Fig. 1. XRD graphs of pure and 3% Co-ZnO NRs

FESEM and EDX analysis

Information on the shape and size of the particles of the manufactured yields was offered by the acquired FESEM pictures. According to the findings shown in Fig 2, pure ZnO has a predicted dimension of 80 nm, whereas the size of doping particles increased after the incorporation of Co to the crystalline structure of ZnO. The XRD results connected this discovery with the larger ionic radius of cobalt atom in comparison with that of zinc atom. This fig indicates that the growing amount of doped cobalt across the volume of ZnO caused the stimulation of a lengthwise development in the particles. Based on the information in fig 2, it was determined that pure ZnO NRs had a height and width of 500 0.2 nm and 100 5 nm, respectively, and that 3% Co-ZnO NRs had a height and width of 1 0.2 m and 50 10 nm. Our nanorods' EDX results validated cobalt's proper introduction during the production of ZnO NRs. As shown in Fig 3, the proportions of cobalt in pure and 3% Co-ZnO NRs were 0 and 2.75%, respectively. These results suggested that there were no contaminants in the structure

Cytotoxic performance

The remarkable benefaction of nanotechnology in the progress of achievements and enhancements of modern strategies for cancer treatment is undeniable [5]. The distinctive physical features of metal nanoparticles resulted in their recognition as appealing applicants which are zinc oxide nanoparticles [10]. This work implicated the cytotoxic evaluation of synthesized pure and 3% Co-ZnO NRs, obtained through by *P. fracta,* in opposition to breast cancer MCF7. For this purpose, the exposure of cells was provided for 24 h at various volumes (1–1000 µg/mL) of pure and doped ZnO NRs by the utilization of WST-1 assay (Figure 4). The data of Figure 4 indicates the exhibition of comparable toxicity impacts by doped NRs to that of pure nanorods, while it is noteworthy that an extension in the rate of doping concentrations led to the induction of notable toxicity effects. As it was observed, the cytotoxicity results were heightened by enlarging the amount of applied concentration, while the significant volume was reported at 1000 µg/mL. These outcomes approved the cytotoxic viability of nanorods on cancer MCF-7 cells.

A large number of authors acclaimed the fascinating impacts of zinc oxide NPs, which were achieved through green synthesis, on cancer cells. The study attempted to examine the anticancer functionality of ZnO nano-powders, which were arranged by utilizing a solution combustion approach through the application of bio fuels *Punica granatum* and *Tamarindus indica*. Considerably, the green synthesized ZnO

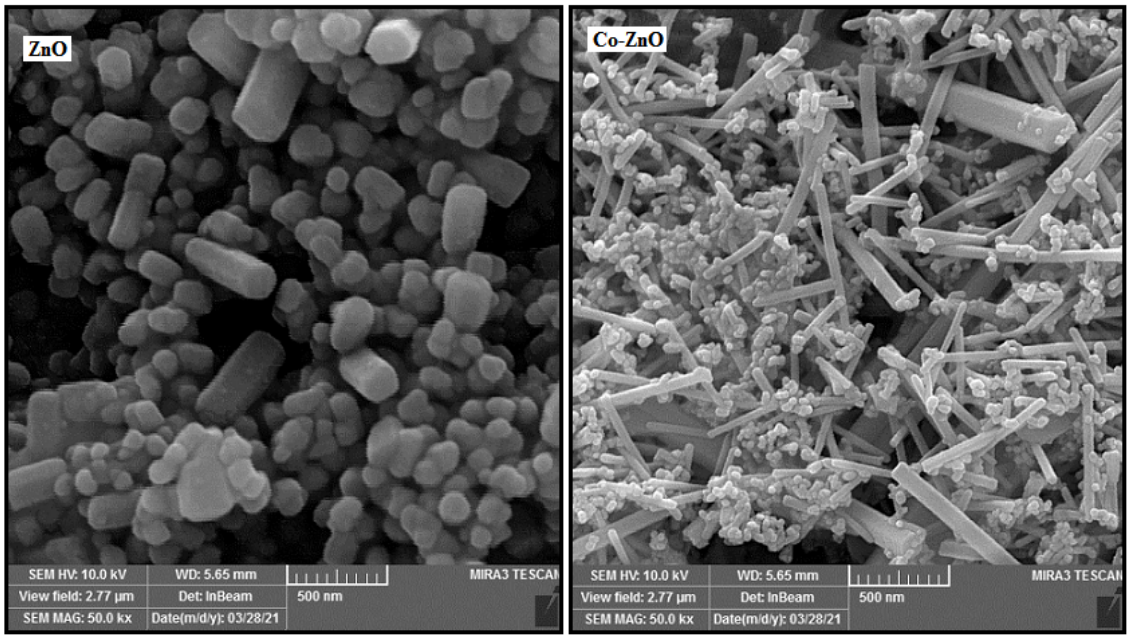

Fig. 2. FESEM images of pure and 3% Co-ZnO NRs

nanopowders succeeded to attain viability values in the highest applied volumes of this work (100 µg/mL), which is quite considerable since commercial ZnO nano-powder can only reach a cell viability of about 66% [27-30].

Breast cancer (BC) is the result of abnormal and unchecked malignant cells multiplying in the breast tissue. The third-leading cause of death worldwide and the second-most prevalent malignancy in women is BC. Surgery, radiation, and chemotherapy are used as adjuvant and neoadjuvant treatments in the treatment of BC [9]. Chemotherapy is a method that uses chemicals to kill cancerous cells. Although it is the most successful method for treating cancer, these chemotherapy drugs' cytotoxic actions cause a number of negative effects [10]. Death from cancer and relapse risk are both reduced with radiotherapy. However, it frequently exposes nearby organs to radiation, raising the risk of pulmonary and cardiac conditions. These treatments may raise the risk of blood cancer, particularly when combined with specific types of adjuvant chemotherapy [11]. However, due to their negative impact on healthy tissues and organs, these therapeutic techniques are frequently ineffective in the treatment of BC.

The absence of selectivity in this therapy represents a serious drawback. Cancer nanomedicine, a multidisciplinary discipline that focuses on the design and medical applications of materials and technology at the nanoscale (usually up to 100 nm), has considerably advanced the creation of cancer treatments during the past few decades [12, 13]. Increased biocompatibility, efficient administration, and diminished or eradicated negative effects are just a few of the possible advantages of nanoparticulate-based delivery methods [14].

With safeguarding from in vivo chemical-based or biological hazards, nanomedicine provides numerous benefits over traditional cancer therapies. These benefits include decreased medication the decomposition throughout transport, improved

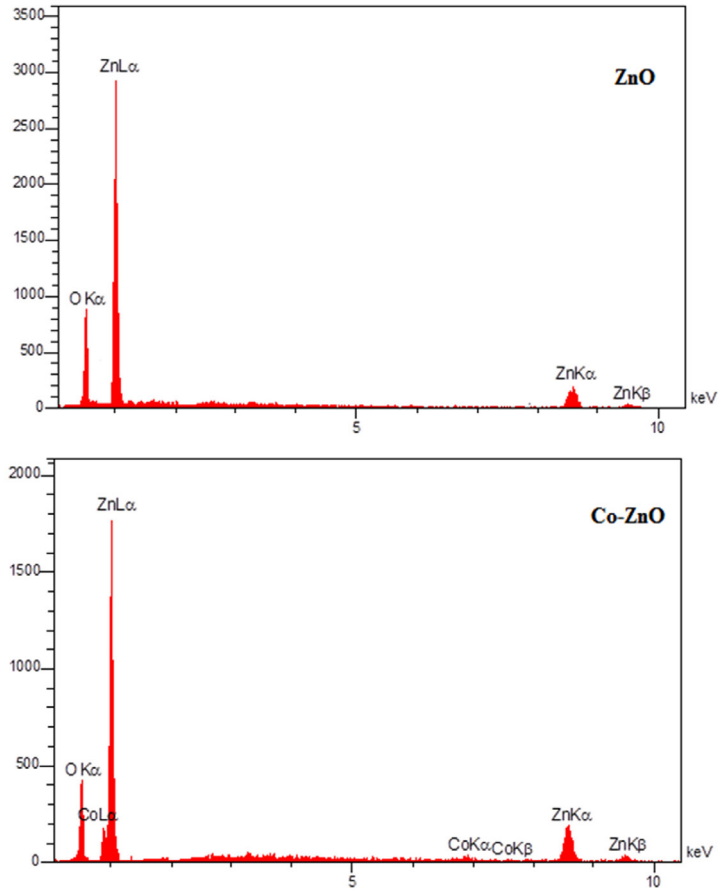

Fig. 3. EDX image of pure and 3% Co-ZnO NRs

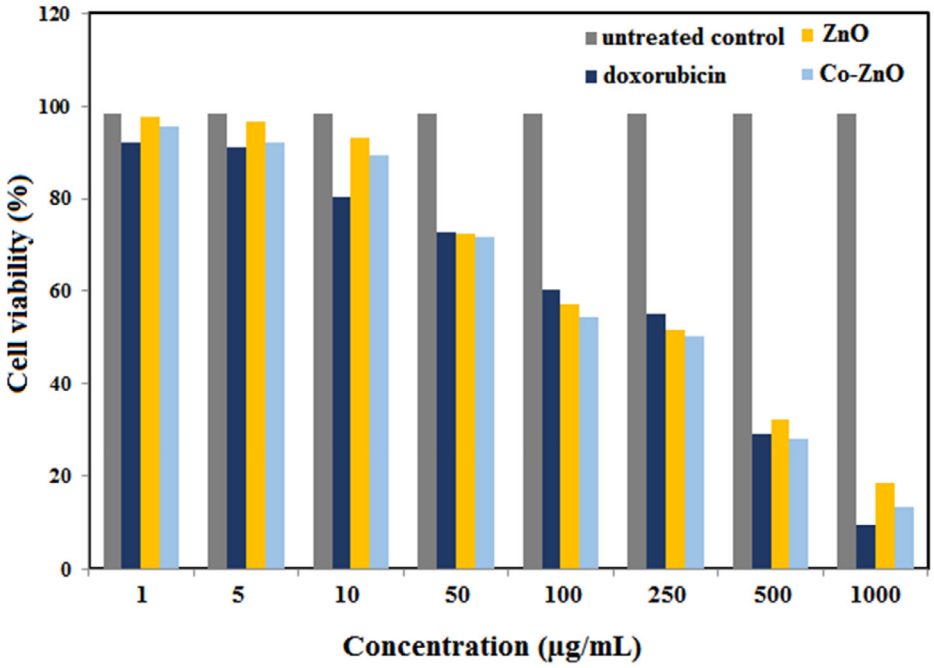

Fig. 4. Cell viability of pure and 3% Co-ZnO NRs on MCF-7 cell line after 24 h incubation.

biocompatibility, and increased chemotherapeutic dosage enforced on cancerous tissue [15]. Additionally, nanomedicine has plenty of promise to effectively mark and eradicate BC stem cells, which may serve a crucial role in the development, recurrence, and resistance to chemo- and radiation in BC. The FDA has approved numerous nanoparticulate-based chemotherapeutic delivery platforms or they are now undergoing clinical trials to treat cancer. This section goes into great length about the paclitaxel-nanoparticle albumin-bound (nabTM) and polymeric nanoparticle-based formulations for treating breast cancer [16].

Cancer diagnosis and therapy may be modernized by cancer nanotechnology. New nanoscale targeting tactics have been created thanks to advancements in material research and protein engineering, giving BC patients new hope [17]. By thoroughly penetrating tumors, pharmaceutical vehicles known as nanoparticles (NPs) create a new point of entry for the delivery of drugs to cancer cells with high levels of selectivity. Additionally, NP therapy reduces its adverse impacts on normal tissues and organs. The National Cancer Institute has given its approval to nanotechnology and regards it as an outstanding paradigm-shifting strategy for bettering BC diagnosis and therapy [18].

CONCLUSION

The Prosopis fracta plant extract turned out to be the best option for performing the successful synthesis of pure and Co-ZnO NRs. The results of laboratory testing tools and methods of analysis, which authorized the production of uniform and imperfectly spherical nanoparticles that were synthesized at the nanoscale, were used to evaluate and identify the physico-chemical properties of this material. After speeding up the doping of cobalt into ZnO, an expansion in nanorod length and diameter was seen. Following that, data from the WST-1 experiment on the harmful effects of nanorods on the MCF7 cell line showed that doped nanorods had a greater inhibitory effect than pure nanorods. As a noteworthy fact, the cytotoxicity of ZnO NRs was observed to be empowered by extending the percentage of applied cobalt into the construction of nanorods. In conformity to the presented facts, one can affirm the viability of manufactured nanorods for being implicated in biological fields and cancer treatment and also approve their stance as a potent and cost-effective choice for the annihilation of environmental pollutants.

Since BC prevalence has increased substantially in the years, along with its rates of morbidity and mortality, it is imperative to provide the most

effective avoiding strategies, keeping in mind that risk factors that may be modified to considerably decrease BC prevalence.

The most popular screening procedure to date that allows for a relatively early identification of BC is mammography and sonography. The treatment and clinical results of BC patients have considerably improved as a result of the ongoing quest for prognostic biomarkers and targets for possible biological therapeutics.

NP-based synthetic materials are currently used for medication administration because they enable adequate oversight over the physicochemical nature of the nanoproducts. The delivery of therapeutic BC medicines to the tumor sites is enhanced by the stability, safety, biological inertness, and prolonged circulation of NP-based synthetic compounds. The production of a corona of plasma proteins close to the surface can be induced by the NPs. As a result, the mononuclear phagocyte system consumes highly charged NPs more quickly than neutrally charged NPs. As a result, the hydrophobicity and surface charges of NPs can be appropriately altered using synthetic nanocarriers, increasing their half-life in circulation. Additionally, their surface properties can be easily modified to enhance conjugation to the intended receptors.

CONFLICT OF INTEREST

There is no conflict of interest

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