Research Article

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Eco-synthesis and characterization of titanium nanoparticles: Testing its cytotoxicity and antibacterial effects

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Abstract: In recent years, green synthesis of nanoparticles has been the cornerstone of development of nanotechnology and its applications, as it emphasizes on promoting environmental sustainability. The aim of the present study was to investigate the potential health benefits of the green-synthesized titanium nanoparticles (TiO₂NPs). TiO₂NPs were synthesized using titanium(IV) isopropoxide and lupin bean extract. The synthesized particles were characterized to assess the average particle size by dynamic light scattering,

and X-ray diffraction method was used to study the crystalline nature. The average particle size recorded was 9.227 nm with a polydispersity index (PDI) of 0.382. The morphology of the particles was assessed by scanning electron microscope and transmission electron microscopy which showed varied shapes of the nanoparticles, uniform spherical and crystallite rod shaped. Further, the cytotoxic efficacy of the nanoparticles was assessed against the breast cancer (MCF-7) cell line using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide for (MTT) assay. The antibacterial activity of the nanoparticles was evaluated against clinical pathogens via the disc diffusion assay. The key findings showed that the TiO₂NPs exhibited potent cytotoxicity against the MCF-7 cell line with an IC₅₀ of 41.1 µg. It also showed profound antibacterial activity. Thus, the synthesized nanoparticles could have potential biomedical applications owing to their therapeutic efficacy.

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1 Introduction

Nanoscience is an emerging research field with wide applications in electronics, material and biomedical sciences. Several metal nanoparticles (NPs) synthesized by varied modes have been extensively reported for their applications in the above-mentioned fields [1]. Despite the numerous advantages of metal NPs, it is imperative to understand the characteristics of different transition metals and their interactions in the biological system [2].

TiO₂ production is on a steep rise worldwide due to its applications in various products such as sunscreens, paints, electronics, solar cells, in addition to sensor technology, environmental remediation and other related fields [3,4]. Several studies report that owing to their high photocatalytic efficiency, TiO₂ materials could play a potential role in antibacterial applications and act as a photosensitizer for the

photodynamic therapy (PDT) in cancer. It has been postulated that TiO_2 exerts a cytotoxic effect against cancer cells and some pathogenic organisms such as fungi and bacteria by the generation of reactive oxygen species (ROS) [5,6].

Thus, titanium oxide nanoparticles (TiO_2NPs) are precious semiconducting transition metal oxide material with excellent magnetic, optical and electric properties [7]. They have certain defining characteristics such as being cost-effective in comparison to noble metals, nontoxic and resistant to chemical erosion. TiO_2NPs have also been reported to have unique physicochemical properties in comparison to their fine particle analogs which may alter their bioactivity [8]. In recent years, plant-based synthesis of NPs has garnered attention in the field of nanotechnology as it is facile, safe, quick, biocompatible and eco-friendly [9,10]. Recent studies have reported the use of various plant species such as *Sesbania grandiflora* L. leaf extract [2], *Moringa oleifera*, *Aloe barbadensis*, *Calotropis gigantea*, *Ageratina altissima* L., *Curcuma longa* and *Vitex negundo* in the synthesis of TiO_2NPs [11–16].

The present study is a continuation of our previous studies on noble metal NPs and their antimicrobial potency [17,18]. Keeping in mind the premise, the current study describes a novel mode of biosynthesis of highly stable, cost-effective and environmentally benign TiO_2NPs crystal using the *Lupinus* (lupin bean) extract. The anticancerous and antibacterial efficacy of the NPs was also assessed. To the best of our knowledge, this is the first study describing the preparation of TiO_2NPs using the lupin bean extract.

2 Materials and methods

2.1 Preparation of the *Lupinus* extract

The *Lupinus* (Lupin bean) powder was procured from the local market. Ten grams of lupin bean powder was soaked in 20 mL deionized water overnight. Thereafter, the solution was filtered to prepare the lupin extract.

2.2 Eco-synthesis of TiO_2NPs

A volume of 20 mL of the prepared lupin bean extract was mixed with 2.5 mM of titanium(IV) isopropoxide (Merck Co., Germany) at a molar ratio of 2:1 under vigorous and continuous stirring at room temperature. On stirring the contents, a white paste started forming at the bottom of the flask, which indicated the formation of TiO_2NPs . The paste

was heated on a hot plate at 80°C and then calcined in a muffle furnace at 450°C for 3 h, producing a beige powder including TiO_2NPs .

2.3 Characterization of eco-synthesized crystal TiO_2NPs

An extensive characterization of the synthesized bio-crystal TiO_2NPs was employed using various techniques. The crystal structure and crystalline grain size were determined by X-ray diffraction (XRD) (Bruker D8 Discover, UK). The average particle size was analyzed by a Zeta-sizer, (Nano series, HT Laser, ZEN3600 from Malvern Instrument, UK). Transmission electron microscopy (TEM) (JEM-1011, JEOL, Japan) was used to study the size distribution and shape. Scanning electron microscopy (SEM) (JEOL-FE, Japan) was employed to characterize the shape and morphology of the formed NPs. Energy dispersive spectrometer (EDS) (Oxford Instrument, UK) was used to characterize the elemental composition (Ti and O) of NPs.

2.4 Cytotoxicity assay

The cytotoxic effect of the synthesized NPs was assessed against the MCF-7 cell line (human breast cancer cell line), obtained from VACSERA Tissue Culture Unit. The cell culture was propagated in Dulbecco's modified Eagle's medium enriched with 10% heat-inactivated fetal bovine serum and 1% L-glutamine 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer; gentamicin was added at a concentration of 50 µg/mL to avoid contamination. The cell culture was maintained at 37°C in a humidified atmosphere supplemented with continuous aeration of 5% CO_2 and was subsequently subcultured twice a week. Cell toxicity was evaluated by determining the effect of the test samples on cell structure and viability. For cytotoxicity assay, the cells were seeded in a 96-well plate at a cell concentration of 1×10^4 cells/well in 100 µL of growth medium. A fresh medium containing different concentrations of the test sample was added after 24 h of seeding to avoid depriving the cells of nutrients. Serial twofold dilutions of the test compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multi-channel pipette. Microtiter plates were incubated at 37°C in a humidified incubator with 5% CO_2 for a period of 48 h. Three wells were used for each concentration of the test sample. Vinblastine (a known anticancer drug) was used as a positive control for the MCF-7 cell line. Control cells were incubated without the test sample and with or without dimethyl

sulfoxide (DMSO). The concentration of DMSO was kept low (maximal 0.1%) and was found to have negligible effect on the course of the experiment. Post incubation of the cells for 24 h at 37°C, different concentrations of the test sample (TiO₂NPs) (100, 50, 25, 12.5, 6.25 and 3.125 µg) were added and incubated for another 48 h, and the yield of viable cells was determined by colorimetric method. After incubation, medium was aspirated, and the crystal violet solution (1%) was added to each well for at least 30 min for color development. The plates were rinsed with tap water until all excess stains were removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly. After gentle shaking, the absorbance of the plates was read on a microplate reader (TECAN) at 490 nm. All results were calibrated, and the corrected absorbance was calculated. Treated samples were compared with the control in the absence of the tested sample. Internal triplicates for each concentration were included in each experiment. Finally, the cytotoxic effect of each tested concentration was calculated [18].

2.5 Antibacterial activity of TiO₂NPs

The bactericidal effect of eco-synthesized TiO₂NPs was investigated using disc diffusion method [19] against *Enterococcus* (Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria). Nutrient agar medium plates were prepared, sterilized and solidified. Bacterial cultures were swabbed on these plates after solidification of the agar medium. Using hole-punched agar plates, the TiO₂NPs solutions at different concentrations (5, 10, 15 µg/mL) were placed in the inoculated nutrient agar plates and kept for further incubation at 37°C for 24 h. The inhibitory potency of the NPs was visualized by the measured zones of inhibition. The experiments were conducted in triplicates, and the mean value of the measured diameter of the inhibition zone was recorded.

3 Results and discussion

3.1 Characterization

Figure 1 shows X-ray diffractograms of TiO₂NPs synthesized using the lupin bean extract. The XRD pattern shows sharp peaks 2θ at about $\approx 25^\circ$, 37° and 48° indicating the existence of crystallite sized particles, with a semicrystalline nature. These results are in agreement with the previous study by Hudlikar *et al.* [20]. All the peaks confirmed the crystal anatase form [21].

Figure 1: XRD pattern of lupin TiO₂NPs.

The dynamic light scattering (DLS) Zetasizer was used to determine the average size of the eco-synthesized NPs. The Z-average mean diameter (d [nm]) of the green TiO₂NPs was 9.227 nm, and the polydispersity index (PDI) was 0.382 with intercept 0.244, with fairly stable condition. However, the peak strongly indicates that the particles are monodispersed as shown in Figure 2. Overall, the size and PDI of the eco-synthesized NPs revealed good stability. These results validated the results of XRD discussed earlier.

TEM is the most frequently reported technique to analyze the morphology and structure of the synthesized NPs. TEM illustrated the shape of the synthesized NPs as shown in Figure 3(a and b); it indicates uniform, almost spherical (Figure 3a) and crystallite rod-shaped particles (Figure 3b) with uniform and narrow size distribution. These electron micrographs of the synthesized TiO₂NPs further confirmed the results of the XRD. Thus, the titanium was formed as a small-sized NP using the lupin bean extract as a directing agent.

The biopolymer, on being dispersed in the liquid media, formed an organic matrix with titanium ions being bound to its functional groups (hydroxylic or carboxylic groups). The association of titanium ions with polysaccharide created a uniform and homogenous dispersion of the ions in the available matrix. Thus, these binding sites provided nucleation centers and growth sites for the hydrolyzed Ti⁴⁺-containing particles, as a result of increased local supersaturation in titanium ions. The oxide precursor requires heating to be changed into oxide ion. The homogeneous dispersion of titanium cations within the matrix of the polysaccharide coupled with low temperature shifted the reaction from nucleation toward the increased crystal growth of the nanooxide particles [22,23].

Figure 2: DLS measurement of lupin TiO_2 NPs.

The subnanostructure and morphology of the TiO_2 NPs were assessed using SEM. The image showed relatively spherical shape and crystallite rods of the green-synthesized NPs (Figure 4a). The EDS results shown in Figure 4b confirm the presence and existence of TiO_2 NPs in suspension. The spectrum analysis revealed signals in the titanium and oxygen regions, which confirmed the formation of TiO_2 NPs, and the table represents the percentage of elements present in the Ti and O_2 suspension.

3.2 Evaluation of cytotoxicity and antibacterial activities of the green TiO_2 NPs

The green NPs had a significant dose-dependent effect on the cell viability as observed in the MTT assay. The cytotoxic effect against MCF-7 cells with IC_{50} of $41.1 \mu\text{M}$

was tested using the eco-synthesized TiO_2 crystal NPs. It was found that the cytotoxicity of green TiO_2 NPs increased with increased concentration of NPs and demonstrated a 100% dose-dependent death in the MCF-7 cells. With the increase in the concentration of the sample, the ROS production also increases which in turn explains the oxidative damage caused to the cancer cells [24]. Thus, a greater number of NPs easily enter the cancer cells and generate free radicals which are responsible for the destruction of the cancer cells [25]. A similar pattern of results on the cell cytotoxicity of NPs was reported by Maheswaria et al. [26], Murugan et al. [27] and Sukirtha et al. [28]. In line with previous *in vitro* studies indicating that the TiO_2 NPs are toxic to the mammalian cells [29], the results of the present study provide evidence of a profound cytotoxic effect of synthesized TiO_2 NPs against the breast cancer MCF-7 cell line in comparison to Vinblastine. Moreover, the size of the nanoparticle and the cell type dominantly affected the cytotoxicity of NPs. Thus, a comparison of the results of the cytotoxicity of the green TiO_2 NPs with those of Vinblastine showed that the NPs were more efficacious (Figure 5).

Figure 6 shows the antibacterial activity of TiO_2 NPs, which was evaluated via the disc diffusion method, against two bacterial strains: *Enterococcus* (Gram-positive) and *E. coli* (Gram-negative). The antibacterial effect of the NPs was visualized by the wide zone of inhibition around the TiO_2 NPs-integrated discs. The basic mechanism of the antibacterial activity stems from the interaction between the positively charged surface of NPs with the net negative charge on the bacterial cell wall [30]. The differential adhering of the positively

Figure 3: (a and b) TEM images of lupin TiO_2 NPs.

Figure 4: (a) SEM image. (b) EDS analysis results presented the percentage of TiO_2 element in green nanosuspension.

charged NPs to the bacterial strains, *Enterococcus* and *E. coli*, is due to the difference in the composition of the cell wall of the Gram-positive and Gram-negative bacteria. It has been reported that the magnitude of the negative charge varies in different strains. The Gram-negative bacteria (*E. coli*) has higher negative charge and adheres more strongly to positive surfaces than the Gram-positive bacteria (*Enterococcus*). This is attributed to the fact that the cell wall of the Gram-negative bacteria consists of an outer membrane composed of lipopolysaccharides followed by a thin layer of peptidoglycan, whereas the Gram-positive bacteria has only a thick layer of peptidoglycan. Thus, the positively charged eco-synthesized TiO_2 NPs are

more active against the Gram-negative bacteria, regardless of their resistance level [31]. Based on previous literatures, it can be hypothesized that an electromagnetic attraction between microorganisms and metal oxides leads to oxidation and eventually the cell death of microorganisms [32]. Overall, the bactericidal effect of the tested NPs could also be attributed to the decomposition of bacterial outer membranes by ROS, primarily hydroxyl radicals (OH), which leads to phospholipid peroxidation and oxidative cell death [33,34].

The above-discussed findings of the present study showed a significant antibacterial effect of the eco-synthesized TiO_2 NPs, particularly against opportunistic

Figure 5: Cytotoxic activity of the green TiO_2 NP suspension compared with that of Vinblastine against MCF-7 cell line.

Figure 6: Antibacterial activity of the green TiO_2 NPs and the values of inhibition zones (mm).

infections caused by multidrug-resistant Gram-negative organisms. Thus, it offers a vast potential for NPs in biomedical applications and cosmetology [35,36].

4 Conclusion

The findings of the study suggest a successful “green” synthesis of environmentally benign TiO₂NPs with the phytoconstituents of lupin bean extract. The bean extract used played a pivotal role in the reduction and stabilization of NPs. The synthesized green TiO₂NPs demonstrated significant cytotoxicity against the breast cancer MCF-7 cell line. Additionally, the NPs also exhibited potent antibacterial activity against both Gram-negative and positive strains. Therefore, NPs offer potential therapeutic applications in the fields of nanomedicine and biomedical science.

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Conflict of interest: The authors declare that they have no conflict of interests.

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