Probe into DNA interaction, cell toxicity and antifungal activities of phyto-synthesized yttrium oxide (Y2O3) nanoparticles

Rugmani Meenambala*, Hema S Kb, Vedika Tomarc, Anita Puyamd

aDepartment of Clinical Psychopharmacology and Neurotoxicology, National Institute of Mental Health and Neurosciences, Bangalore, India
bDepartment of Bio-Sciences, Vellore Institute of Technology, Vellore, India
cAmity Institute of Nanotechnology, Amity University, Noida, India
dDepartment of Plant Pathology, Rani Lakshmi Bai Central Agricultural University, Jhansi, India

*Corresponding Author: m rugmani@yahoo.com

Abstract Green synthesis is considered to be eco-friendly approach in comparison to chemical mediated synthesis and hence the study demonstrates facile Nyctanthes arbor-tristis mediated phyto-synthesis of biocompatible yttrium oxide (Y2O3) nanoparticles. X-ray diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR) were employed to confirm the formation of cubic Y2O3 nanoparticles. Further, the interaction of Y2O3 with calf thymus-DNA were scrutinized by UV-visible, fluorescence spectroscopy and agarose gel electrophoresis techniques and the results suggested the stabilization of CT-DNA by the biosynthesized Y2O3. Also, the synthesized Y2O3 does not exhibit any toxicity in SK-MEL, human melanoma cell lines. Further, the studies on antifungal activities of Y2O3 nanoparticles revealed significant inhibitory effects on the growth of plant pathogen Exserohilum rostratum causing Exserohilum leaf blight in pearl millet. Hence, the findings deliver the foundation for further biological research investigations of Y2O3 and its application in phytopathogen control.

Keywords: Phyto-synthesis, yttrium oxide, DNA interaction, antifungal activity

1. Introduction

The development of metal oxide nanoparticles has received attention in biomedical applications owing to larger surface area, magnetic and optical characteristics, antioxidant and antimicrobial activities, mechanical stability and biocompatibility. Yttrium oxide (Y2O3) is of substantial interest owing to their antioxidant and radical scavenging activity under physiological conditions. A recent study demonstrated that Y2O3 nanoparticles encumbered with a biomaterial scaffold can induce angiogenesis and vascularization with its potential application in tissue engineering [1]. Y2O3 nanoparticles have been prepared using different synthesis techniques but most of these techniques suffer from shortcomings and therefore, bio or green synthesis stands an advantage by being less toxic, economic, environmentally friendly and can be processed for large-scale production [2]. Although few studies report phytosynthesis of yttrium oxide nanoparticles, the potential plants extract as biological materials for the synthesis of nanoparticles is yet to be explored [3]. In the present study, the leaf extract of the plant, Nyctanthes arbor-tristis is used as stabilizing and capping agents in the synthesis of Y2O3 nanoparticles. The extracts of the plant are reported to act as immune enhancer and hepatoprotective agent along with antimicrobial and anti-parasitological properties in vitro[4].
To the best of our knowledge, there are no other reports on *N. arbor-tristis* mediated phytosynthesis of yttrium oxide nanoparticles. The studies on the interaction of nanoparticles with biomolecules including DNA is well explored owing to the likely effects that the nanoparticles bring about in various molecular mechanisms such as synthesis, replication and structural integrity of nucleic acids [5,6]. It is significant to study the molecular interactions as the interacted nanoparticles may alter the properties of DNA and also results in the conformational change of DNA, which in turn affects gene expression, replication, repair, transcription and signalling mechanism [7]. Hence, nanoparticle-DNA interaction is considered as one of the most promising field of investigation for molecular and targeted therapies. The present study demonstrates *N. arbor-tristis* mediated phytosynthesis of yttrium oxide nanoparticles along with its material characterization. The interaction of Y2O3 nanoparticles with calf thymus DNA were also assessed with spectroscopic techniques and agarose gel electrophoresis. The cytotoxic effect of the nanoparticles on SK-MEL viability was assessed using MTT assay. Further, their antifungal properties against *Exserohilum rostratum* isolated from pearl millet were examined.

2. **Materials and Methods**

2.1 **Phyto-synthesis and characterization of Y2O3 nanoparticles**

The leaves of *Nyctanthes arbor-tristis* were collected, processed and the crude extract was prepared in deionized water. The stock solution containing 0.1 M and 0.5M yttrium nitrate hexahydrate was added dropwise to 10ml of leaf extract under continuous stirring conditions at room temperature. The homogenous suspension was then kept in a hot air oven at 120 °C overnight and the dried samples were calcined at 400 °C in a muffle furnace and used for further characterization. The phase purity and composition of Y2O3 nanoparticles were analysed using powder X-ray diffractometer (Rigaku, Ultima IV, Japan) with scan range of 20 between 10° and 60°. For determining the functional groups, the transmittance of the prepared samples was recorded using a FTIR spectrophotometer (Perkin–Elmer, USA) in the scan range of 4000- 400cm⁻¹ in the infrared region.

2.2 **Effects of DNA interaction with nanoparticles**

A stock solution of CT-DNA (10 µg/ml) were prepared in 10 mM Tris HCl buffer with mild quaking until the formation of homogenous solution mixture which was maintained at a pH of 7.4 and the resultant mixture was stored at 4°C. DNA interaction with nanoparticles was performed by treating nanoparticles of different concentration (20 and 40 µg/ml) with 10µg ofCT-DNA [6]. All the samples including the control without nanoparticles, and suitable blank were utilized to check the interaction of Y2O3 nanoparticles with DNA through UV spectrophotometry within the wavelength region of 200-400 nm with constant DNA concentration and steady state fluorescence assay with emission spectrum scanned at 350 to 650 nm.

2.3 **Agarose gel electrophoresis studies**

The cleavage activity of samples was tested through agarose gel electrophoresis technique [8]. In a reaction mixture of 20 µl, 3200 µM of calf thymus DNA was treated with different concentrations of Y2O3 nanoparticles. Both the control DNA and Y2O3 conjugated DNA were
exposed to agarose gel electrophoresis embedded in 1% gel in 0.5 % TAE buffer for 1h. The resultant gel was imaged using the GELSTAN 4X Advanced gel-documentation system.

2.4 Cytotoxicity test

All the samples and controls were seeded with fibroblast line SK-MEL, human melanoma cell lines during 24 hours, in Dulbecco's modified eagle's medium (D-MEM) accompanied with fetal bovine serum (FBS) and penicillin-streptomycin. The viability of the Y2O3 treated cells in comparison with control cells was evaluated using an Inverted phase contrast microscope (Labomed TCM-400 with MICAPS™ HD camera) and was further quantified by MTT assay by measuring absorbance at 570 nm using an ELISA plate reader [9].

2.5 Antifungal test

Two concentrations of 0.1M and 0.5M were used for the study and potato dextrose agar plates were divided into two parts, A (control) and B (Y2O3 nanoparticles) which were inoculated 1mm away from the edge of the plate. The growth rate was recorded on alternate days for both the parts and the test was performed in triplicates. Antagonistic activity was checked after incubation by measuring the radial growth rate of Exerholium rostratum in Y2O3 treated plates (R2) and in the control plate (R1). Further, the percentage inhibition of radial growth (PIRG) was determined using Skidmore and Dickinson formula [10]:

\[
PIRG = \frac{(R1 - R2)}{R1} \times 100
\]

Further, to see the impact of the particles on fully grown fungal mycelium, 0.16 mg of the Y2O3 nanoparticles were sprinkled over the culture plate that have been inoculated three days before and another plate of same incubation period was kept as control without any treatment.

3. Results and Discussion

Yttrium oxide (Y2O3) nanoparticles were successfully synthesized through Nyctanthes arbortristis mediated phyto-synthesis route. The powder X-Ray diffraction (XRD) patterns as shown in Figure 1a corresponds to crystalline yttrium oxide prepared by phytosynthesis route which was calcinated at 400 °C and all resultant peaks were in good ordinance for a cubic structure of Y2O3 (ICDD: 01-071-0049). FTIR peak were recorded for stretching of Y-O bond and bending of C-O at 566 cm⁻¹ and 674, 854 and 1381, 1525 cm⁻¹ respectively [11]. FTIR spectra of the calcinated samples also intense peak at 566 cm⁻¹ due to Y-O stretching [12]. Both the results from XRD and FTIR spectra are in good agreement with earlier literature reports.

The absorption spectra of various concentrations of Y2O3-DNA with respect to constant concentration of CT-DNA (10 µg/ml) is depicted in the Figure 2a. A maximum absorption at 260nm is witnessed in the samples owing to the electronic transitions from components of DNA molecules such as purine and pyrimidine [13]. With increasing concentration of Y2O3, the samples exhibited a significant red shift, offering that there exists a strong interaction between the Y2O3 nanoparticles with CT-DNA. In steady state fluorescence, the emission spectrum of Y2O3 nanoparticles exhibited a sharp emission peak at 510 nm at an excitation wavelength of 260 nm. A hypsochromic shift of photoluminescence spectrum is observed with enhanced luminescence intensity with increasing concentration of Y2O3 that indicates interaction of Y2O3 with DNA.
Figure 1. (a) XRD patterns (b) FTIR spectra of Y$_2$O$_3$ samples heat treated at 400 °C

Figure 2. (a) UV-Visible absorption spectra and (b) Steady state fluorescence spectra of CT-DNA along with different concentration of Y$_2$O$_3$ nanoparticles

Figure 3 shows the gel electrophoretogram of CT-DNA with different concentration of Y$_2$O$_3$ nanoparticles which was subjected to a run time of 60 min at100V. 3200µM of CT-DNA was maintained as control as shown in the first lane of Figure 3. Further, significant mobility of DNA was observed in lanes 1 to 4 on addition of varying concentrations of Y$_2$O$_3$ in CT-DNA. The mobility might be an indication of binding between CT-DNA and Y$_2$O$_3$ nanoparticles [14].

It is important to check the biosafety of developed nanoparticle prior to its biological applications and here MTT assays were performed to validate cell viability. Dose dependent decrease in cell viability was detected in SKMEL cancer cells with the administration of different concentrations of the sample labelled as Y01. The IC 50 value was observed with 24.95 µg concentration of this test sample.
The IC 50 value of YO2 was observed with 57.75 µg concentration of this test sample. After sample addition, the treated as well as the control wells were observed at regular intervals up to 24hrs in an inverted phase contrast tissue culture microscope and the observations were photographed. Although no cytotoxic effects were observed at lower concentration of Y2O3 nanoparticles, higher concentrations exhibited toxic behaviour which is attributed to over-accumulation of particles inside the cell. The observed minor changes in the structure of the cells, including girdle, shrinkage, agglomeration, and vacuolization in the cytoplasm were treated as pointers of toxicity [15].

**Figure 3.** Agarose gel of control and CT-DNA at different concentrations of Y2O3 nanoparticles from lane 1 to 4 respectively

Further, in the antifungal test there was variation in growth rate in the treated plates as compared to untreated control. It was found that YO2 was found more effective as compared to YO1. Although in case of YO1, the inoculum could initiate its growth. 0.16 mg of YO2 was used for conducting the experiment further and it was found effective. It completely restricted the growth of the pathogen (Figure 5). The radial colony on part B of the treated plate completely lie in the origin when plotted on the graph as indicated in Figure 5 and the growth on part A of the same plate was greatly reduced as compared to part A and B of the control plate. This clearly indicates the nanoparticle in used has antifungal property. Percent inhibition zone ranges from 51.50 to 60.53% in three replicates with an average of 56.39%. It could be stated that Y2O3 nanoparticles might inhibit the growth of *Exserohilum rostratum* by pervading the fungal cell and destructing the cell wall and cellular mechanisms, thereby restricting the mycelial growth [16].
Figure 4. (a) Cell viability of different concentration of Y2O3 nanoparticles in SK-MEL, human melanoma cell lines. Microscopic images of (b) control, (c) 6.25µg/ml YO1 and (d) 6.25µg/ml YO2 nanoparticles incubated for 24 hours

Figure 5. (a) Graph showing the difference in Radial growth of Exserohilum rostratum on potato dextrose agar. Antagonistic effect of Y2O3 nanoparticles on Exserohilum rostratum culture plates marked with part A and B (a) control and (b) treated
4. Conclusion

Yttrium oxide (Y2O3) nanoparticles have successfully synthesized using aqueous leaf extract of Nyctanthes arbor-tristis and the results from X-ray diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR) confirm the formation of cubic Y2O3. The UV–Visible absorption and steady state fluorescence studies suggested the interaction of CT-DNA with Y2O3 nanoparticles. The mobility of gel detected through electrophoresis might be an indication of binding between CT-DNA and Y2O3 nanoparticles. Further, the toxicity tests showed that Y2O3 nanoparticles does not show cytotoxic effects at 24 hours of exposure in SK-MEL, human melanoma cell lines at lower concentrations. The results also demonstrate the potential antifungal activity of Y2O3 nanoparticles have good antifungal activity against Exserohilum rostratum. Therefore, the results obtained from this study can be used for studying various mechanisms underlying biological applications. 

Acknowledgments

The financial assistance received from Department of Science and Technology (DST) [Reference: [DST/INSPIRE/04/2018/000925], India is acknowledged. The authors declare that there are no conflicts of interest.

References

[1] Augustine R, Dalvi Y B, Nath V Y, Varghese R, Raghuveeran V, Hasan A, Thomas S and Sandhyarani N 2019 Yttrium oxide nanoparticle loaded scaffolds with enhanced cell adhesion and vascularization for tissue engineering applications Mater. Sci. Eng. C. 103 109801.

[2] Das R K, Pachapur V L, Lonappan L, Naghdi M, Pulicharla R, Maiti S, Cledon M, Dalila L M A, Sarma S J and Brar S K 2017 Biological synthesis of metallic nanoparticles: plants, animals and microbial aspects Nanotechnol. Environ. Eng. 2 1-21

[3] Kannan S K and Sundrarajan M 2015 Biosynthesis of Yttrium oxide nanoparticles using Acalypha indica leaf extract Bull. Mater. Sci. 38 945-50

[4] Ilyas U, Katare D P, Aeri V and Naseef P P 2016 A review on hepatoprotective and immunomodulatory herbal plants Pharmacogn Rev. 10 66–70

[5] Ribeiro A P C, Anbu S, Alegria E C B A, Fernandes A R, Baptista P V, Mendes R, Matias, A S, Mendes M, da Silva M G and Pombeiro A J L 2018. Evaluation of cell toxicity and DNA and protein binding of green synthesized silver nanoparticles Biomed. Pharmacother. 101 137-144

[6] Babu E P, Subastri A, Suyavaran A, Rao P L, Kumar M S, Jeevaratnam K and Thirunavukkarasu C 2015. Extracellularly synthesized ZnO nanoparticles interact with DNA and augment gamma radiation induced DNA damage through reactive oxygen species RSC Adv. 5 62067-77
[7] An H and Jin B 2012. Prospects of nanoparticle–DNA binding and its implications in medical biotechnology Biotechnol. Adv. 30 1721-32

[8] Singh A and Kaushik M 2019. Physicochemical investigations of zinc oxide nanoparticles synthesized from Azadirachta Indica (Neem) leaf extract and their interaction with Calf-Thymus DNA Results Phys. 13 102168

[9] Meenambal R and Kannan S 2018. Design and structural investigations of Yb3+ substituted β-Ca3 (PO4) 2 contrast agents for bimodal NIR luminescence and X-ray CT imaging Mater. Sci. Eng. C. 91 817-23

[10] Schoeman M W, Webber J F and Dickinson D J 1996 The effect of diffusible metabolites of Trichoderma harzianum on in vitro interactions between basidiomycete isolates at two different temperature regimes Mycol. Res. 100 1454-58

[11] Krishna R H, Nagabhushana B M, Nagabhushana H, Chakradhar R P S, Sivaramakrishna R, Shivakumara C and Thomas T 2014 Auto-ignition based synthesis of Y2O3 for photo- and thermo-luminescent applications J. Alloys Compd. 585 129–37

[12] Shivaramu N J, Lakshminarasappa B N, Nagabhushana K R and Singh F 2016 Synthesis characterization and luminescence studies of gamma irradiated nanocrystalline yttrium oxide Spectrochim. Acta A Mol. Biomol. Spectrosc. 154 220–31

[13] Nagababu P, Kumar D A, Reddy K L, Kumar K A, Mustafa M B, Shilpa M and Satyanarayana S2008 DNA binding and photocleavage studies of cobalt(III) ethylenediamine pyridine complexes: [Co(en)2(py)2] 3+ and [Co(en) 2(mepy)2]3+ Metal-Based Drugs 100 275084

[14] Das S, Chatterjee S, Pramanik S, Devi P S and Kumar G S 2018 A new insight into the interaction of ZnO with calf thymus DNA through surface defects J. Photochem. Photobiol. B: Biol. 178 339–47

[15] Miller M A and Zachary J F 2017 Mechanisms and Morphology of Cellular Injury, Adaptation, and Death In Book Pathologic Basis of Veterinary Disease., ed Miller M A and Zachary J F (Elsevier) Chapter 1 p.2

[16] Kumari M, Giri V P, Pandey S, Kumar M, Katiyar R, Nautiyal C S and Mishra A 2019 An insight into the mechanism of antifungal activity of biogenic nanoparticles than their chemical counterparts PESTIC BIOCHEM PHYS 157 45–52