Pritha Chakraborty et al /J. Pharm. Sci. & Res. Vol. 8(11), 2016, 1253-1257

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Bioactivity of Lanthanum Nanoparticle Synthesized using *Trigonella foenum-graecum* Seed Extract

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Abstract

Aim: Green synthesis of nanoparticles include natural plant, fruit, leaf or seed extracts and can be used for medicinal applications. Lanthanum nanoparticles have been applied in various fields. In this study, fenugreek seed aqueous extract was used to synthesize lanthanum nanoparticles.

Methods: The size and characteristics of the nanoparticles were determined by scanning electron microscope (SEM), FTIR analysis. Antibacterial activity against selected clinical pathogens was explored. Antioxidant activity and anticancer activity was also checked against osteocarcoma cell line (MG63).

Result and conclusion: Result shows synthesized lanthanum nanoparticle poses moderate antibacterial activity and excellent antioxidant and anticancer activity.

Key words: Green synthesis, Lanthanum nanoparticle, Fenugreek seed extract, Antibacterial activity, Osteocarcoma.

INTRODUCTION

Nanoparticle has multifunctional properties with applications in various fields such as medicine, nutrition and energy [1]. The biogenic synthesis of monodispersed nanoparticles with specific sizes and shapes have been a challenge in biomaterial science. There are many ways to synthesize nanoparticles such as solid reaction, chemical reaction, and sol gel method etc [2]. The growing need of environment friendly nanoparticles has attracted lots of researchers to use green synthesis methods with a variety of metal nanoparticles [3] due to their interesting, attractive and remarkable properties with a variety of applications over their bulk material [4, 5]. In this study, the plant extract has been used as reducing agent and capping agent for the synthesis of nanoparticles due to their reducing properties [6].

Trigonella foenum-graecum (fenugreek) is a self pollinating annual leguminous bean which belongs to Fabaceae family, commonly known as Indian methi, It is one of the most ancient medicinal herbs [7]. Fenugreek seeds are the most important and useful part of fenugreek plant. The fenugreek, plant mainly shows the presence of saponin and alkaloids.

Because of their unique electronic configuration [4f electrons] lanthanides have been applied in various fields; also these lanthanide-based materials have attractive and interesting magnetic [8], optical electrical and therapeutic properties [9, 10]. Among the lanthanides, lanthanum has been extensively examined for its unique properties.

The current investigation focuses on the aqueous extract of fenugreek seeds to synthesize lanthanum nanoparticles using different experimental conditions and exploring the bioactivity of synthesized nanoparticle.

MATERIALS AND METHODS

Chemicals: Lanthanum nitrate (LaN_3O_9) was purchased from SigmaAldrich. Deionised distilled water was used throughout the experiment. All other chemicals were of analytical grade.

Preparation of the extract: Fenugreek seeds were purchased from local shop near VIT University, Vellore. Seeds are washed with tap water and dried in hot air oven at 50°C. The clean and dried seeds are crushed into coarse grounded powder. 10 gm seed powder was added to 150 ml distilled water in a conical flask and boiled at 100°C for 15 mins. This extract was filtered and stored at 4°C for further investigations.

Preparation of Lanthanum Nitrate solution: 10 ml of 1mM lanthanum nitrate solution was freshly prepared using sterile distilled water.

Synthesis of Lanthanum nanoparticles

For the synthesis of nanoparticles, 10ml of 1mM lanthanum nitrate solution was added drop wise to 3ml of fenugreek seed extract and kept in shaking condition at room

temperature. After one hour, 10ml of methanol was added and kept undisturbed at room temperature for 30 mins. The precipitate formed was separated by centrifugation at 4500 rpm for 15 mins at 4°C and collected in eppendorf tubes and dried in hot air oven at a temperature of 45°C [11].

Characterization Of Nanoparticle

Scanning electron microscope (SEM)

The morphology of the lantunaum nanoparticles was observed by field emission scanning electron microscopy (FE-SEM) (AMRAY1910) equipped with a backscattered electron detector at 15–30 kV. For SEM images, the samples were sputter-coated with about 15 nm Au using a Polaron coater system [12].

FT-IR (Fourier Transform Infrared Spectroscopy)

Infrared (IR) spectra of the synthesized nanoparticle were recorded at room temperature in the frequency range of 4,000–400 cm−1 with a fourier transform infrared (FTIR) spectrophotometer (8400 Shimadzu, Japan, with Hyper IR-1.7 software for Windows) with a helium–neon laser lamp as a source of IR radiation. Pressed pellets were prepared by grinding the extracted samples with potassium bromide in a mortar with 1:100 ratio and immediately analyzed in the region of 4,000–400 cm−1 at a resolution of 4 cm^{-1} [13].

Bioactivity Of Nanoparticle

Antibacterial activity

The antibacterial activity of lanthanum nanoparticle against nine bacterial pathogens was evaluated by using agar well diffusion method. Muller Hinton Agar (MHA) plates were inoculated with selected bacterium. Wells of 8 mm size were made with sterile borer on agar plates. Four different volumes (25 µl, 50µl, 75 µl, 100 µl) of the plant extract were poured into each well of inoculated plates. Then they were left at room temperature for ten minutes allowing the diffusion of the plant extract into the agar. After incubation for 24 hrs at 37°C, the plates were observed for clear zone. Antibacterial activity of the nanoparticle was identified by an inhibition zone surrounding the well containing the plant extract. The zone of inhibition was measured and expressed in millimeters [14].

Antioxidant Activity

The antioxidant activity of the synthesized nanoparticle was evaluated by DPPH radical scavenging assay which was originally described by Blois [15]. DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a synthetic free radical with deep violet colour when is in form of solution which has a λ_{max} at 517 nm. It can accept an electron or hydrogen radical to become stable diamagnetic molecule and appear as light purple in colour which indicates the scavenging of DPPH and the substance has antioxidant activity.

Nanoparticle solutions were prepared with suitable solvent. Methanol solution of DPPH was used as negative control. 500µl of each sample and 500 µl of DPPH solution was allowed to react and incubated at room temperature for 30mins under dark conditions. Absorbance was taken at λ_{max} i.e. 517nm against a blank which was 500µl of methanol. Percentage inhibition was calculated by the following equation to conclude the presence of antioxidant activity of the extracts.

Percentage of inhibition

= (OD control – OD sample / OD control) x 100

Anticancer study

Cell line

The human osteosarcoma cell line (MG 63) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37°C, 5% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37°C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved in dimethylsulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37°C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples served as control which was maintained in triplicates for all concentrations.

MTT assay:

After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then the absorbance was measured at 570 nm using micro plate reader.

The percentage of cell viability was then calculated with respect to control as follows

Percentage of cell viability = [A] Test / [A]control x 100

The percentage of cell inhibition was determined using the following formula.

Percentage of cell inhibition

= 100- Abs (sample)/Abs (control) x100.

Nonlinear regression graph was plotted between percentage of cell inhibition and Log concentration and IC50 was determined using GraphPad Prism software [16, 17].

RESULTS AND DISCUSSION

Scanning Electron Microscopy (SEM)

The size and surface morphology of the synthesized nanoparticle was examined using SEM. The SEM image of the nanoparticle is shown in Fig 1. From the figure it is very clear that the nanoparticles are almost spherical in shape and uniform in size measuring below 100nm. The surface morphology of the nanoparticle shows that the particles are agglomerated. The distribution of the crystallite sizes is not homogenous. It also indicates the highly crystalline nature of the synthesized particles. From these observations it may be inferred that during the simultaneous precipitation process LaN_3O_9 precipitates first. This process creates active nucleation sites for LaN_3O_9 crystals from the La ions, facilitating crystallization at a much lower temperature [18].

FT-IR

FT-IR result explores the presence of particular functional groups of the synthesized lanthanum nanoparticle (Fig 2). It shows band at 3356.14 which are characteristics of amides (N-H stretch). Bands at 2926.01 and 1423.47 are characteristics of alkanes (C-H stretch). Bands at 1633.71 and 815.89 are characteristics of carboxylic acids (C=O bend). Bands present at 1543.05, 1315.45, 1143.79 1nd 1012.63 indicates the presence of aromatic group (C-C stretch), nitro compounds (N-O symmetric stretch), alkyl halides(C-H wag) and aliphatic amines (C-N stretch) respectively. The presence of different functional groups can be associated with different bioactivity exhibited by the synthesized nanoparticle. The presence of C-H, C-C stretching and C=O bend confirms the agglomeration of the nanoparticles [19].

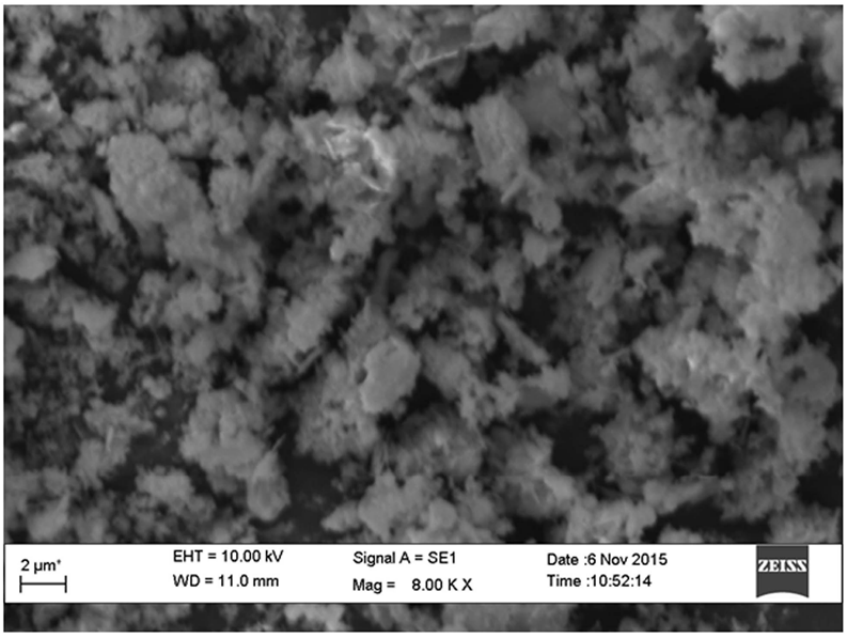

Fig 1: SEM image of lanthanum nanoparticle from *Trigonella foenum-graecum* seed extract.

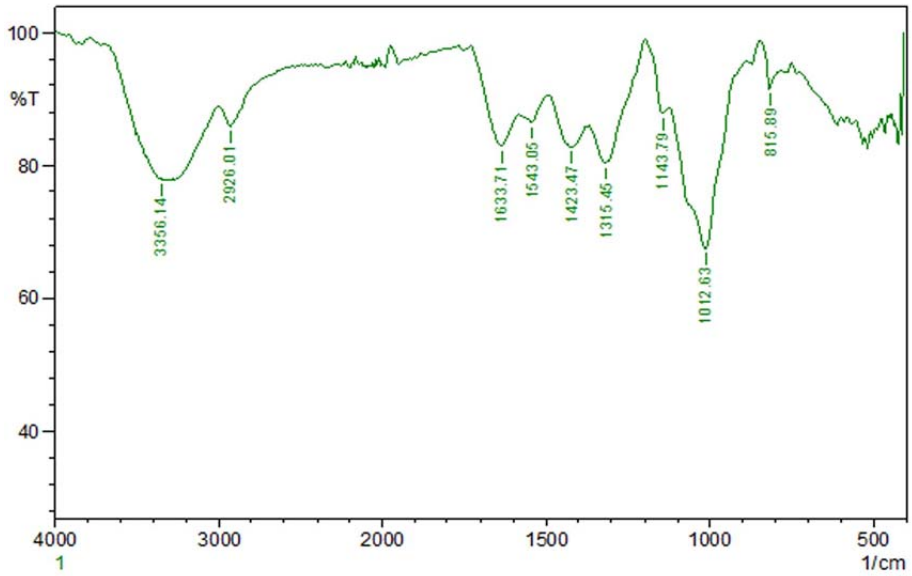

Fig 2: FT-IR image of lanthanum nanoparticle from *Trigonella foenum-graecum* seed extract.

Table 1: Antimicrobial activity of lanthanum Nanoparticle from *Trigonella foenum-graecum* seed extract.

.	Strains	Zone of inhibition			
		25mg/ml	50mg/ml	75mg/ml	100mg/ml
1	*Pseudomonas aeruginosa*	0.7	0.9	1.7	2.3
2	*Serretia* sp.	0.5	1.2	1.5	2.5
3	*Proteus mirabilas*	-	-	-	-
4	*Shigella dysentaria*	-	-	-	-
5	*Staphylococcus aureus*	-	-	-	-
6	*Klebsiella* sp.	1.0	1.2	1.3	1.8
7	*Enterobacter* sp	-	-	-	-
8	*Salmonella* sp.	-	-	-	-
9	*E.coli*	-	-	-	-

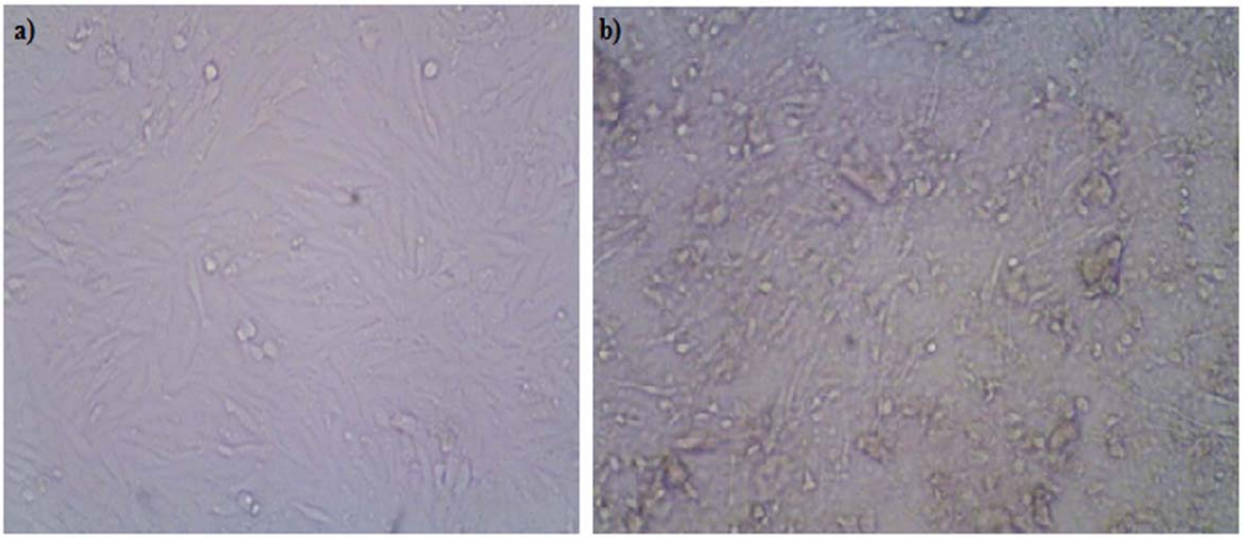

Fig 3: Anticancer activity a) control, b) test of lanthanum nanoparticle from *Trigonella foenum-graecum* seed extract against MG 63 cell line

Antibacterial activity

Antibacterial activity of lanthanum nanoparticle was checked against eight clinical pathogens which was determined by measuring zone of inhibition formed after incubation period. No inhibition zone was observed against six test pathogens which are *Eschericia coli*, *Salmonella* sp., *Shigella* sp., *Staphylococcus aureus*, *Proteus mirabilus*, *Enterobacter* sp. Pronounced clear zone was observed against *Pseudomonas aeruginosa*, *Klebsiella* sp and *Serretia* sp. among nine test pathogens [20]. The zone of inhibition was very pronounced. Highest zone of inhibition was observed at 100mg/ml concentration against *Serretia* sp. Table 1 indicates the result of the antimicrobial activities of lanthanum nanoparticle.

Antioxidant activity

The antioxidant activity of the nanoparticle was evaluated by DPPH free radical scavenging assay which was originally described by Blois. DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a synthetic free radical with deep violet colour when in form of solution has a λ_{max} at 517 nm. The radical scavenging potential of the antioxidant was measured by the degree of discoloration. The synthesized nanoparticle has shown 60.6% antioxidant activity, which can be considered as moderate activity. The reduction capacity of DPPH free radical was determined by the decrease in its concentration induced by antioxidative compounds. Antioxidantive compound reacts with DPPH and convert it to 1,1-diphenyl-2-picrylhydrazine by donating electron to terminate the radical chain [21].

Anticancer study

Anticancer study of the nanoparticle was observed against human osteosarcoma cell line (MG 63). Anticancer activity of nanoparticle was checked. Cell viability has been found to decrease with increased concentration of nanopaticle, which indicates the moderate activity of the nanopartcle. On the other hand, 1.0 µg/ml concentration of nanoparticle showed less than 20% of cell inhibition while at 2.5 µg/ml concentration of nanoparticle, percentage of cell inhibition reaches 60%, which indicates that percentage of cell inhibition is increased with increasing concentration of the nanoparticle. It indicates the presence of anticancer activity of synthesized lanthanum nanoparticle [22].

CONCLUSION:

In this present study the synthesis of lanthanum nanoparticles was synthesized by biological method using fenugreek seed extract which acts as a reducing agent to reduce lanthanum metal to nanosize particles. Lanthanum nanoparticle has shown moderate antibacterial activity against specific clinical pathogens, antioxidant activity and anticancer activity against osteocarcoma cell line. This proves that the plant mediated nanoparticles have the potential to be used in various fields such as pharmaceuticals, therapeutics and other commercial products.

There is no conflict of interest from the authors.

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