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Biogenesis of Hematite Nanoparticles Employing *Prosopis cineraria* and their Antioxidant Property

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Interaction of green principles and metallic ions is a fascinating field of research to accomplish synthesis of biocompatible nanoparticles. In present study, Fe_2O_3 nanoparticles were fabricated utilizing phytochemical of wonder tree, *Prosopis cineraria* a natural herbal infusion. The leaf constituents of *P. cineraria* acted as reductant and stabilizer. The UV-vis spectrum showed characteristic SPR at 270 nm depicts the formation of iron oxide nanoparticles. Fourier transform infrared (FTIR) spectroscopy suggested that primary carboxylic acid, esters have involved in the synthesis. Further high-resolution transmission electron microscopy (HR-TEM) revealed that the newly formed nanoparticles are square shaped with an average size of 24 nm. Field emission scanning electron microscopic (FESEM) studies depicts their smooth surface without agglomeration. EDAX analysis divulges the elemental composition, Fe, O, Cl, C, K and Ca. The X-ray diffraction (XRD) studies revealed their crystalline phase. Toxicological responses were investigated using *in vivo* studies with experimental fish *Cirrhinus cirrhosus*, depicts that the assessed antioxidant enzymes catalase (CAT), glutathione-S-transferase (GST) and superoxide dismutase (SOD) did not show impact and the mortality was recorded only at higher (2200 mg kg^{-1}) concentration.

Keywords: Iron oxide nanoparticles, Biological synthesis, Wonder tree, Carboxylic acid, Esters, Biocompatible.

INTRODUCTION

Nanomaterials offer tremendous predictions for research and technological development in diverse areas pertaining to global concern. It is a fast growing transdiciplinary field of science and technology with increasing advances in medical applications which includes imaging, diagnostics, drug delivery, therapeutics [1-3]. Applications of nanoscience have expanded due to the convenient surface bioconjugate with significant optical properties concomitant localized plasmon vibration [4], frequency of surface plasmon vibration the visible spectral range and shape of the particle environment depend on a number of factors, such as quantitative dielectric properties and particle interactions [4,5].

Currently, research has focused on developing methods aimed at producing biocompatible nanoparticles that do not use toxic substances [6]. Iron oxide nanoparticles having prospect to open new boulevard in medical science using atomic scale tailoring of materials. It progresses at burgeoning phase of innovative research making influence in all spheres of human lifestyles. The present investigation addresses green chemistry principles on synthesizing iron oxide nanoparticles is fascinating due to its biocompatible nature. FeO nanoparticles are also effective due to its attainability, strength and large surface area. In addition, its extensive range of utilization in technology *viz.*, sensors, magnetic storage, biomedicine, catalysis, thin films, pharmaceutical distribution, *etc*. [7-9].

Green synthesis provides better influence, control over crystal growth and their stability [10-12]. As the environmental exposure of nanomaterials increases, it has become imperative to assess the degree of their toxicity. Proper evaluation of the toxicological properties of nanoparticles is very important, especially if it is to be applied in the medical sciences [13].

Rational selection of phytochemically active biological molecules to activate the surface of these particles will certainly increase biocompatibility. Nowadays, medicinal plants play an important role in the treatment of infectious diseases and they are readily available and inexpensive compared to synthetic compounds. In this work, the Fe_2O_3 nanoparticles using *Prosopis cineraria* leaf extract as reducing and stabilizing agent were synthesized and characterized.

EXPERIMENTAL

Fresh young leaves of wonder tree, *Prosopis cineraria* were collected from the Temple at Thiruvalam, Vellore, India. Ferric chloride was procured from Himedia Laboratories Pvt. Ltd., India. Deionized water was prepared in the laboratory and used throughout the experiment.

Fresh leaves (100 g) were washed thoroughly then cut into small pieces and crushed with 10 mL of distilled water using mortar and pestle. The fine colloidal extract was made up into 100 mL with distilled water and then filtered using Whatman No. 1 filter paper. As a result of various experimental conditions and concentration of mixture 60 mL of stock solution of leaf extract with 40 mL of ferric chloride (0.1 M) was found to synthesize Fe_2O_3 nanoparticles. Reaction was monitored under UV-visible spectroscopy, after 6 h of reaction the solution was start to became dark brown and continued till 9 h. It was then centrifuged at 5000 rpm for 15 min. The pellets were collected and used for characterization.

Characterization of Fe_2O_3 nanoparticles: The UV-visible spectrum was recorded on the Shimadzu UV-2300. The X-ray diffraction (XRD) analyses were performed at 40 kV with JCPDS No. 96-101-1241 and Cu$K\alpha$ radiation at a current of 30 mA. The Fourier Transformer Infrared (FTIR) spectra in the 4000-400 cm^{-1} ranged was analyzed using Perkin- Elmer FTIR spectrophotometer. The morphology of nanostructures was recorded using the Hitachi S4800 field emission scanning electron microscope (FESEM) for energy transmissible X-ray analysis (EDAX), whereas high resolution transmission electron microscopy (HRTEM) micrographs were obtained using JEOL 3010 running at 120 kV.

Animal and acute toxicity assessment of Fe_2O_3 nanoparticles: The experimental animal freshwater fish, (Mrigal carp) *Cirrhinus cirrhosus* (80-90 g weighed and 18-20 cm length) irrespective of sex were collected from Vellore Fort Moat, India. Fishes were acclimatized for 7-10 days to the laboratory conditions. The laboratory temperature was maintained at 28 ± 2 ºC and the illumination was set at 12 h light and 12 h dark. The synthesized Fe_2O_3 nanoparticles acute toxic effect was determined on *C. cineraria*. The experiments were performed for 7 days and the lethal concentration (LC_{50}) was measured with five different concentrations (1500, 1600, 1800, 2000, 2200 mg kg^{-1}) of Fe_2O_3 nanoparticles. At these concentrations, the mortality rate (LC_{50}) was detected at 2200 mg kg^{-1} of Fe_2O_3 nanoparticles for further experimental studies. Blood samples were collected after experimental conditions, further muscles, liver and gills were dissected out to analyze the level of antioxidant enzymes.

Antioxidant enzyme activity: Blood samples were centrifuged at 4000 rpm for 15 min and the collected plasma fractions were separated for each analysis. Then, the blood cells were hemolyzed with distilled water and DNA-se (1 mg/mL) and the cells were frozen overnight at 80 ºC. The hemolyzate fractions were again centrifuged and the hemolyzate fractions were separated for each analysis. Samples of hemolyzate were be frozen and stored at -80 ºC until used. Catalase (CAT; EC 1.11.1.6) was analyzed for its activity by measuring a decrease in peroxide concentration at 240 nm with functions [14]. The activity of glutathione-S-transferase (GST) was analyzed according to Ozaslan *et al.* [15] method. The reaction started after preheating the reaction mixture to 37 ºC for 5 min, after adding 0.1 mL of crushed tissue and 0.1 mL of molecular weight glutathione. Absorption was monitored at 340 nm for 3 min and the enzyme-free reaction solution was subjected as blank. The activity of GST was recorded as moles of GSHCDNB conjugate min^{-1} mg^{-1} protein. Superoxide dismudase (SOD; EC 1.15.1.1) activities were measured by superoxide radicals using xenon/xanthan oxidase by the ferricitochrome C method. The enzyme activity of the current analysis can be reported in SOD units per milligram Hb or protein. A unit of activity is defined by the amount of enzyme required to inhibit the ferricitochrome C reduction rate of 50% [16].

Statistical evaluation: Statistical treatment subjected one way ANOVA, where F values indicated significance, the means were compared by a post hoc multiple range test.

RESULTS AND DISCUSSION

UV-visible studies: The synthesis of Fe_2O_3 nanoparticles has been confirmed by UV-visible spectral analysis (Fig. 1). A characteristic peak of surface plasmon resonance was observed at 270 nm, which was attributed to the formation of Fe_2O_3 nanoparticles [17].

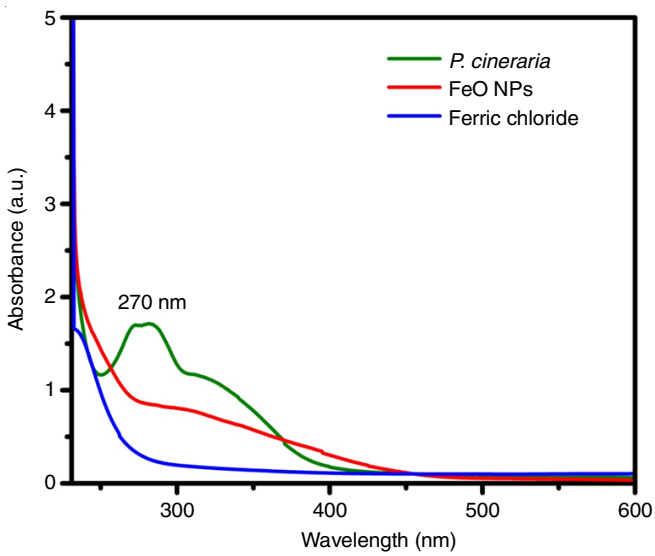

Fig. 1. UV-vis spectrum recorded reduction of ferric chloride with aqueous leaf extract of *Prosopis cineraria*

Morphology studies: The sample of nanoparticles newly synthesized was subjected to HR-TEM analysis. The results indicated that Fe_2O_3 nanoparticles are square in structure and the aggregation was evident (Fig. 2). The average size of the particles is 24 nm or less in size and 85% of the particles are nearly 24 nm in size. FESEM analysis revealed that the biosynthesized Fe_2O_3 nanoparticles having smooth surface, without agglomeration of nanoparticles and the newly formed Fe_2O_3 nanoparticles were well dispersed (Fig. 3).

The EDAX spectrum contains intense peak of Fe and O in addition to Cl and C. The Cl signals must be originating from

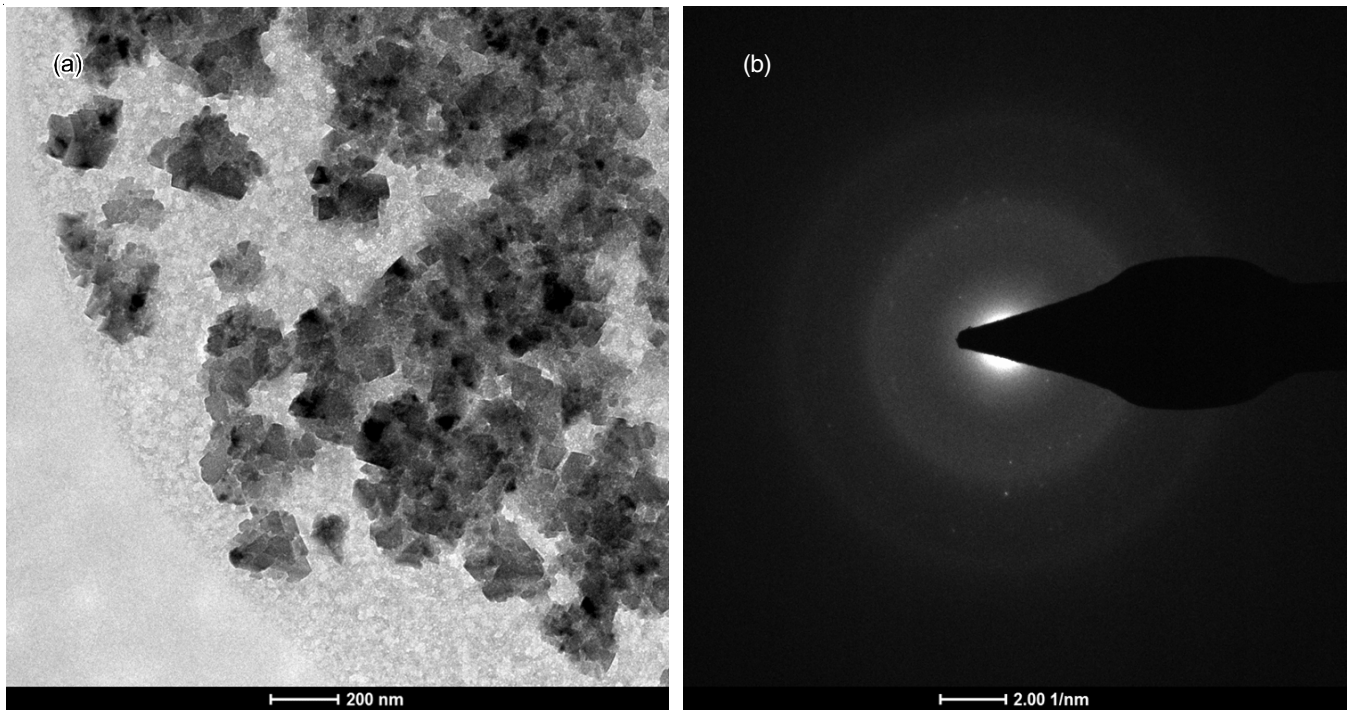

Fig. 2. TEM image (a) and SAED pattern (b) of iron oxide nanoparticles synthesized using aqueous leaf extract of *Prosopis cineraria*

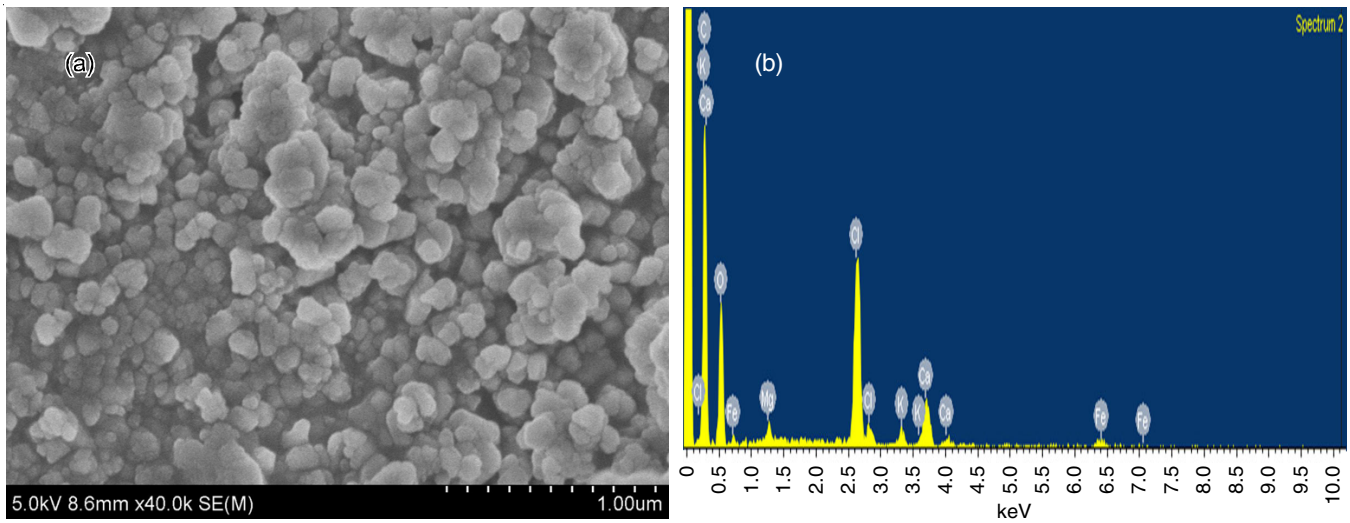

Fig. 3. SEM image (a) and EDAX pattern (b) of iron oxide nanoparticles synthesized using aqueous leaf extract of *Prosopis cineraria*

$FeCl_3$ precursors utilized in the biological synthesis procedure. The C signals are attributed to the organic molecules of *P. cineraria* aqueous leaf extract (Fig. 3b). Kanagasubbulakshmi & Kadirvelu [18] reported the occurrence of such kind of organic molecules, calcium and potassium and pointed that as these compounds interprets the complex of plant extract subjected for synthesis.

FTIR studies: In the FT-IR spectrum of *P. cineraria* leaf extract reveals various functional group peaks at 673 cm^{-1} for –C=C-H: C-H bend, alkynes; 1075 cm^{-1} for -C-O *str*., alcohols, carboxylic acid, ester, ethers, whereas 1401 cm^{-1} corresponds to O-H *str*. due to phenolic groups. The major shift in peak from 1075 to 1145 cm^{-1} validated the formation of hematite nanoparticles which were happened due to the reducing and stabilizing activity of carboxylic acid, esters and alcohols of *P. cineraria* leave extract (Fig. 4).

XRD studies: The XRD pattern discloses the presence of diffraction peak with 2θ values of 24.23º, 33.23º, 35.7º, 49.5º, 54.1º, 62.4º and 64.0º corresponding to the *hkl* values (110), (211),(101), (202), (312), (310) and (211), respectively indicating the crystalline phase of the iron oxide nanoparticles (Fig. 5).

Toxicity studies: In present studies, toxicological responses were conducted using the fresh water fish *C. cirrhosus*. The experimental fish *C. cirrhosus* was reared with different levels (1500, 1600, 1800, 2000, 2200 mg kg^{-1}) of Fe_2O_3 nanoparticles. The leaf extract of *P. cineraria* synthesized Fe_2O_3 nanoparticles exhibits a dose dependent activity and mortality was increased

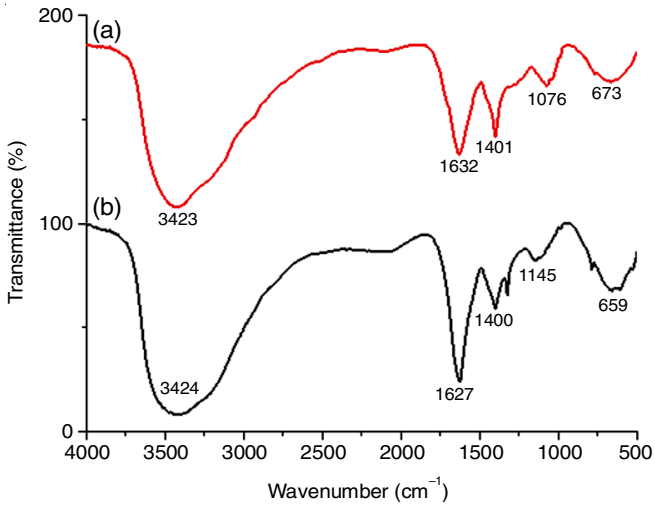

Fig. 4. FTIR spectrum of (a) leaf extract of *Prosopis cineraria* and (b) iron oxide nanoparticles synthesized using *Prosopis cineraria*

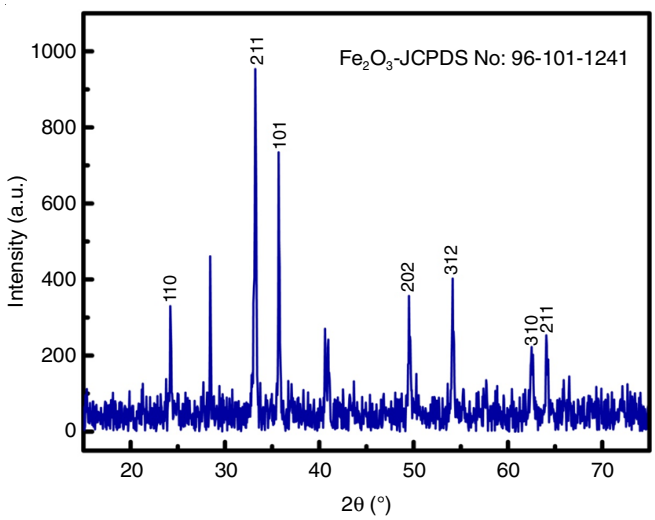

Fig. 5. XRD pattern of iron oxide nanoparticles using leaf extract of *Prosopis cineraria*

with increasing the concentration of Fe_2O_3 nanoparicles. The 100% of mortality was observed at 2900 mg kg^{-1} concentration and no mortality was noticed in the concentrations such as 2000 and 1900 mg kg^{-1} Fe_2O_3 nanoparticles. The LC_{50} was observed at 2200 mg kg^{-1} concentration and no mortality was recorded in control counterparts, during the experimental conditions. Accordingly, further studies on certain enzymes level were analyzed below the concentration LC_{50} value. Due to the fact that antioxidant enzymes would be the most probable indicator of exposure to toxic material, the tissues of control and fishes exposed to nanoparticles were biochemically analyzed to ascertain the toxic nature.

Results discloses that the variation in the level of antioxidant enzymes (CAT, GST, SOD) in *C. cirrhosus* fish during the experimental conditions with of Fe_2O_3 nanoparticles for 7 days is not significant. It is clear that neither Fe_2O_3 nanoparticles cause damage nor influence the metabolic activities of the experimental organism (Fig. 6).

Toxic effects on iron oxide nanoparticles on larval stages of *A. salina* at different doses (0, 25, 50, 100, 200, 400 and 600 mg/L) were also studied. After exposure to iron oxide nanoparticles, the reactive oxygen species, malondialdehyde content, total antioxidant capacity and activity of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) increased significantly.

The amount, process and time-dependent toxicity of superparagnetic Fe_3O_4 nanoparticles (10 nm, 0-400 g/mL) in primary rat hepatocytes were also examined. The results obtained indicate that the ROS response increased with increasing concentrations of Fe_3O_4 nanoparticles. Inspite of emerging wide applications of nanomaterials in different fields, a large number of investigations have been carried out on the toxicological nature and the results discloses that nanomaterials effects varies according to the physico-chemical properties [19-21].

Conclusion

In this investigation, α-Fe_2O_3 nanoparticles were synthesized *via* green route is promising. Currently hematite nanoparticles are finding their fascinating application on medical science because of their higher chemical stability and biocompatibility. In this context biogenic process to generate α-Fe_2O_3 nanoparticles demonstrated herein gains their significance. Based on the *in vivo* studies using a freshwater fish *C. cirrhosus* it has been concluded that the newly formed α-Fe_2O_3 nanoparticles are non-lethal. The tested concentrations of α-Fe_2O_3 nanoparticles did not cause impact on the antioxidant enzymes and the mortality was observed only at higher concentration. The leaf extract of *Prosopis cineraria* serve a dual role as reductant and capping agent which results in the fabrication of α-Fe_2O_3 nanoparticles in a highly benign manner.

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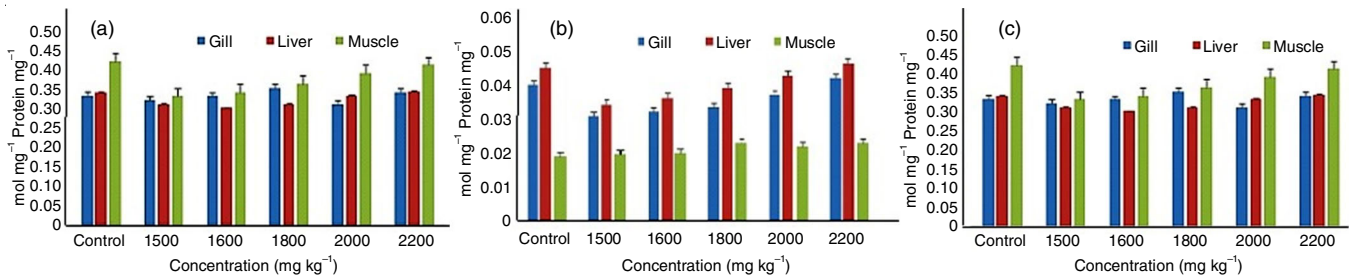

Fig. 6. Toxicity effect of Fe_2O_3NPs on antioxidant enzyme CAT (a) GST (b) and SOD (c) @ *Cirrhinus cirrhosus*

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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