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Biomedical applications of ferulic acid encapsulated electrospun nanofibers

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ABSTRACT

Ferulic acid is a ubiquitous phytochemical that holds enormous therapeutic potential but has not gained much consideration in biomedical sector due to its less bioavailability, poor aqueous solubility and physiochemical instability. In present investigation, the shortcomings associated with agro-waste derived ferulic acid were addressed by encapsulating it in electrospun nanofibrous matrix of poly (D,L-lactide-co-glycolide)/polyethylene oxide. Fluorescent microscopic analysis revealed that ferulic acid predominantly resides in the core of PLGA/PEO nanofibers. The average diameters of the PLGA/PEO and ferulic acid encapsulated PLGA/PEO nanofibers were recorded as 125 ± 65.5 nm and 150 ± 79.0 nm, respectively. The physiochemical properties of fabricated nanofibers are elucidated by IR, DSC and NMR studies. Free radical scavenging activity of fabricated nanofibers were estimated using di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium (DPPH) assay. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay confirmed the cytotoxicity of ferulic acid encapsulated nanofibers against hepatocellular carcinoma (HepG2) cells. These ferulic acid encapsulated nanofibers could be potentially explored for therapeutic usage in biomedical sector.

1. Introduction

Recently, there has been an emerging research interest is noticed in renaissance of naturally occurring phenolic compounds as a potential therapeutic agents against broad range of infirmities including cancer, diabetes, aging, cardiovascular and neurodegenerative disease [1–3]. Among the naturally occurring polyphenolic plant constituent, ferulic acid possess a wide range of biomedical effects including antioxidant, anti-inflammatory, antimicrobial, antiallergic, anticarcinogenic, antithrombotic, antiviral, hepatoprotective, and vasodilatory actions [4–6]. Ferulic acid has also been approved globally as food additive to prevent the peroxidation of lipids as it affectively scavenges the superoxide anion radical [7,8]. However, despite the broad range of bioactivities, the therapeutic applications of ferulic acid are limited due to its poor bioavailability, low water solubility and physiochemical instability in human body fluids [9,10]. The conjugation of such bioactive molecules with biodegradable and biocompatible polymeric matrix, improves the water solubility of drug molecules due to the presence of interactions between drug and polymer matrix. Polymeric matrix provides an environment to the drug in which dispersed drug molecules can reside and experience the intermediate mobility and solubility as compare to pure components [11]. This in turn results into advanced pharmacokinetics properties as well as reduced side effects of drug [12,13]. A number of approaches have been introduced to formulate such systems which consists of biodegradable polymers along with antioxidant compound in order to improve the safety, therapeutic efficacy, stability and reduce toxicity by delivering the drug in a sustained manner for a prolong period [10,14]. In this investigation, we used electrospinning approach to encapsulate agro-waste derived ferulic acid in PLGA/PEO polymeric nanofibrous matrix to combat the shortcomings associated with it. These ferulic acid encapsulated nanofibers were then evaluated for its antioxidant and cytotoxic potential against HepG2 cells.

2. Materials and methods

2.1. Materials

Ferulic acid was isolated from *Parthenium hysterophorus* plant which was locally obtained and its identification was authenticated by Forest Research Institute (FRI), Dehradun, India. The polymers, PLGA (Mw∼45,000) and PEO (Mw∼900,000) were

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purchased from Sigma–Aldrich (St. Louis, MO, US). Dichloromethane (DCM), *N*,*N*-dimethylformamide (DMF), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), cell culture-grade dimethyl sulfoxide (DMSO), phosphate buffer saline (PBS), di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium (DPPH) assay kit, acridine orange (AO), ethidium bromide (EtBr), Dulbecco's Modified Eagle Medium (DMEM) and all analytical grade chemicals were procured from Himedia (India). Human hepatoma cell line (HepG2) was obtained from National Center for Cell Science (NCCS), India.

2.2. Extraction and characterization of ferulic acid

Plant sample (whole plant) was dried under sunlight and kept in oven at 40 °C for 12 h. The dried samples were grounded to powder for the extraction of ferulic acid as described earlier [15,16]. Briefly, 2.0 g powder of plant sample was taken in 250 mL round bottom flask containing 60 mL NaOH (2 M). To prevent the oxidation of ferulic acid during alkali treatment, 0.001 g $NaHSO_3$ was added and kept within rotary shaker at 25 °C, 180 rpm for 24 h. Sample was then centrifuged at 12,000 rpm for 10 min and the obtained supernatant was acidified with by 2 M HCl solution ($pH \leq 2$). Acidified sample was treated three times with 60 mL ethyl acetate and concentrated for the extraction of ferulic acid. The concentrated extract was dissolved in equal volume of acetonitrile/water for further analysis [17]. Ferulic acid bands from TLC plate was scraped and dissolved in 2.0 mL acetonitrile. The samples ($n = 3$) were quantitative analyzed using HPLC (Knauer, Germany) with C_{18} column having mobile phase consisting of acetonitrile/water (80:20) and 0.1% acetic acid and absorbance was taken at 320 nm.

2.3. Preparation of electrospun nanofibers

Polymers (PLGA:PEO:1:1) at an absolute concentration of 2 wt% were dissolved in DCM/DMF (4:1, v/v) solvent to prepare PLGA/PEO nanofibers. A blended solution containing ferulic acid and PLGA/PEO polymers was prepared by dissolving ferulic acid (2 mg/mL) in the above prepared 2 wt% PLGA/PEO polymeric solution. PLGA/PEO and ferulic acid containing PLGA/PEO solutions were then separately loaded into a 5.0 mL syringe which is attached to a metallic needle (21 G). Electrospinning process was performed at a fixed applied voltage (18 kV) and solution flow rate (0.5 mL/h). The fabricated nanofibers were collected on aluminum foil collector, placed at 12 cm horizontal distance from the metallic needle tip. The collected nanofibers were placed in desiccators for 24 h to get rid of any residual solvent.

2.4. Spectroscopic and microscopic analysis

Infrared absorption of isolated ferulic acid, PLGA/PEO and ferulic acid encapsulated PLGA/PEO nanofibers were examined by infrared (IR) spectrometer (Thermo Nicolet Nexus 6700, US) while nuclear magnetic resonance (NMR) experiments were performed using 500 MHz high resolution NMR spectrometer (Avance 500 Bruker Biospin Intl. AG, Switzerland). The phase behavior of samples (10–12 mg) was observed by heating them at 10 °C/min from 25 to 500 °C under the nitrogen atmosphere using a differential scanning calorimeter (DSC; EXSTAR TG/DTA 6300). Morphology of fabricated nanofibers was observed using field emission scanning electron microscopy (FESEM; Quanta 200F Model, FEI, Netherland) at an accelerated voltage of 15 kV. Samples for FESEM were prepared by cutting them into 1 × 1 cm pieces and sputter coated with gold for 1 min using a Biotech SC005 sputter coater. The diameter of nanofibers was measured from FESEM

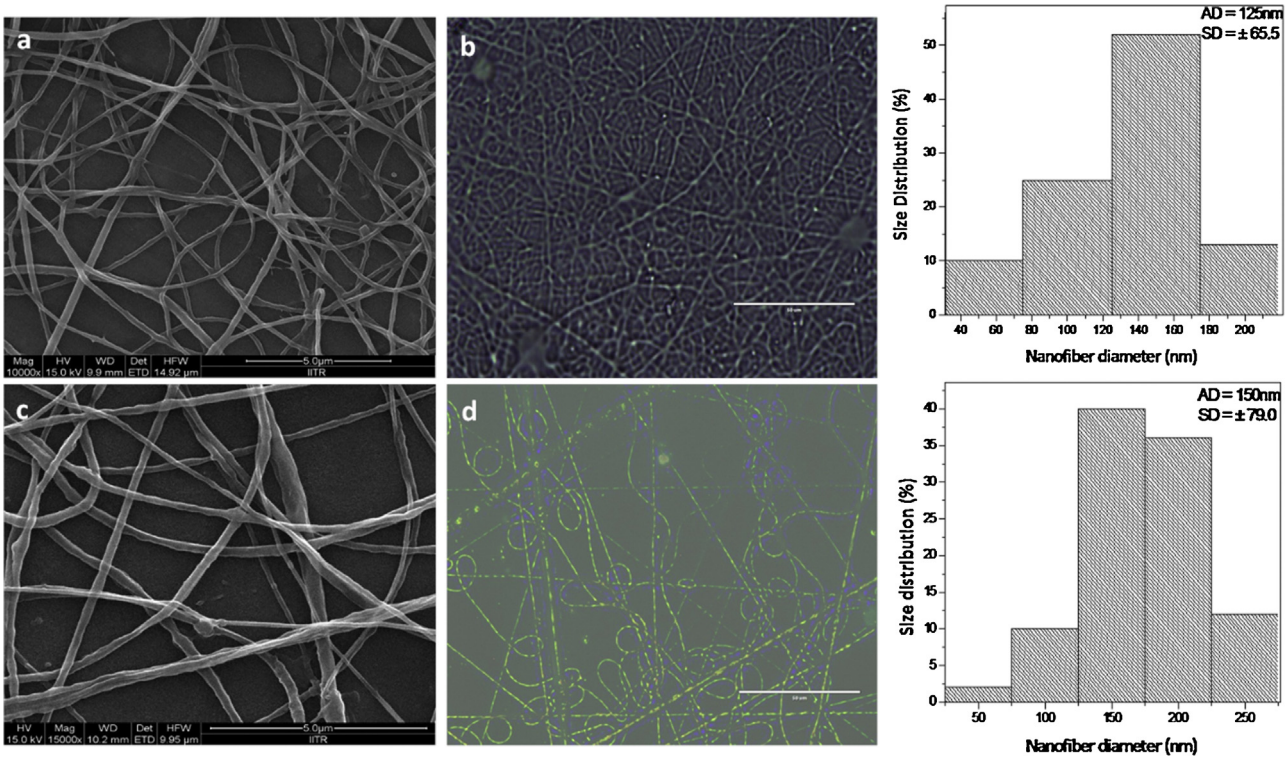

Fig. 1. FESEM and fluorescent micrographs of PLGA/PEO (a and b) and ferulic acid (FA) encapsulated PLGA/PEO (c and d) nanofibers with corresponding diameter distribution histograms.