Journal of Trace Elements in Medicine and Biology 39 (2017) 116–123

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

Microwave-assisted biosynthesis of zinc nanoparticles and their cytotoxic and antioxidant activity

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ARTICLE INFO

Article history:
Received 5 April 2016
Received in revised form 9 August 2016
Accepted 2 September 2016

Keywords:
Microwave-assisted synthesis
Biogenic
Zinc nanoparticles
Lavandula vera
Antioxidant
Cytotoxicity

ABSTRACT

The present study was designed for microwave assisted synthesis of zinc nanoparticles (Zn NPs) using *Lavandula vera* leaf extract in the presence of $ZnSO_4$ (1 mM). The biogenic Zn NPs were then characterized using scanning electron microscopy (SEM), energy dispersive X-ray (EDX), X-ray diffraction spectroscopy (XRD), UV–visible spectroscopy, and Fourier transform infrared spectroscopy (FTIR) techniques. Thereafter, the cytotoxic effect of $ZnSO_4$ and Zn NPs on different cell lines was investigated by MTT-based cytotoxicity assay and their antioxidant properties were assessed using DPPH scavenging activity and reducing power assay. The SEM micrograph showed that the Zn NPs had spherical shape with the size range of 30–80 nm. For A549, MCF-7, HT-29, and Caco-2 cell lines treated with Zn NPs, the concentration necessary causing 50% cell death (IC_{50}) was found to be $22.3 \pm 1.1\ \mu g\ mL^{-1}$, $86 \pm 3.7\ \mu g\ mL^{-1}$, $10.9 \pm 0.5\ \mu g\ mL^{-1}$, and $56.2 \pm 2.8\ \mu g\ mL^{-1}$, respectively. In the case of $ZnSO_4$, the same results (IC_{50}) were observed at concentration of $81.6 \pm 1.3\ \mu g\ mL^{-1}$ (A549), $121.0 \pm 2.4\ \mu g\ mL^{-1}$ (MCF-7), $43.0 \pm 1.4\ \mu g\ mL^{-1}$ (HT-29), and $85.7 \pm 2.3\ \mu g\ mL^{-1}$ (Caco-2). The obtained results of antioxidant activity showed that the IC_{50} values of butylated hydroxyanisole (BHA) and Zn NPs were $44\ \mu g\ mL^{-1}$ and $65.3\ \mu g\ mL^{-1}$, respectively, while $ZnSO_4$ at concentration of $200\ \mu g\ mL^{-1}$ exhibited only 10.9% DPPH radical scavenging effect. Moreover, the reducing power of Zn NPs and BHA were significantly higher than $ZnSO_4$ ($p < 0.05$). To sum up, application of *L. vera* leaf extract combined with microwave heating energy led to simple and fast formation of Zn nanostructures exhibited higher antioxidant and cytotoxic activity compared to soluble Zn^{+2} ions. However, identification of the related mechanisms merit further studies.

1. Introduction

Remarkable applications of metallic nanoparticles in various fields such as medicine, nutrition, and energy due to their unique physicochemical and optical properties introduced this field as a growing trend during the two last decades [1,2]. Special characteristics of zinc (Zn, one of the hardest elements in the group II to VI) nanostructures and its derivatives including i) semiconducting (used in photocatalytic reactions and development of sensors), ii) piezoelectric (the ability of transforming mechanical energy into electric signal or vice versa), iii) ultraviolet shielding (applied in production of laser diodes, LEDs, and photodetectors), and iv) UV-filtering properties (manufacturing of personal care products like sunscreens and cosmetics) make this valuable element as the fourth most widely applied metal in the world after iron, aluminium and copper [3–5]. In addition, the critical role of Zn (as a micronutrient) in the human health through stabilization of DNA structure, participation as cofactor in various vital enzymes like superoxide dismutase (SOD), carbonic anhydrase, and carboxypeptidase, regulation of gene expression, proper function of immune system [6] as well as its antioxidant activity [7,8] has been previously reported by the both in vivo and in vitro studies [9,10]. So, many physicochemical approaches like thermal/laser ablation, sol-gel process, vapor deposition, and chemical reduction etc. have been developed for synthesis of such valuable nanostructures. However, most of

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http://dx.doi.org/10.1016/j.jtemb.2016.09.001
0946-672X/

the mentioned techniques suffer from utilization of sophisticated equipments and/or toxic chemicals and production of environmentally hazardous by-products [11,12]. Therefore, there are increasing attempts to set up simple, cost-effective, and ecofriendly methods for synthesis of such important nanoparticles [13,14]. In this regards, notable advantages of biological entities (such as microorganisms and plant extracts) introduce them as suitable substitutes for nanoparticles assembly [15,16]. Moreover, application of the plant extracts for biosynthesis of various nanostructures is a very simple, fast, and green synthesis method which do not require very expensive and complicated equipments [11,17].

Microwave (MW) is a portion of the electromagnetic spectrum (with frequencies 300 MHz–300 GHz) used as a heating method [18,19]. Different advantages of MW heating compared to conventional bath heating such as uniform heating, very short thermal induction period, selective formation of specific morphology, superheating of solvents over the boiling points of the solvent, and generation of localized high temperatures at the reaction sites make it suitable for reducing the metal ions in solutions [20,21]. Utilization of MW heating methods for preparation of gold, silver, bimetallic, ZnS, and carbon nanomaterials have been previously reported [22–24].

There are many reports about the production of Zn nanowires, Zn nanocomposites, Zn containing quantum dots, and ZnO nanoparticles using physicochemical methods [25,26]. Furthermore, biological synthesis of Zn NPs assisted by plant extracts of *Justicia adhatoda* L. [27] and *Parthenium hysterophorous* [28] were also reported. However, to the best of our knowledge and according to a survey of the literature, there are no reports on biosynthesis of Zn NPs by combination of plant extract (as reducing agent) and MW heating method.

The main aim of the present study was biosynthesis of Zn NPs using *Lavandula vera* leaf extract assisted by MW heating and characterization of the produced nanostructures. In addition, the cytotoxic and antioxidant activities of the prepared biogenic Zn NPs were also evaluated.

2. Materials and methods

2.1. Chemicals and reagents

Zinc sulfate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA), dibasic sodium phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), potassium ferricyanide, trichloroacetic acid (TCA), and ferric choloride ($FeCl_3$) were purchased from Merck Chemicals (Darmstadt, Germany). 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), $AlCl_3$ methanolic solution (10%), Folin–Ciocalteu's reagent, gallic acid, and rutin were supplied by Sigma–Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS), Dulbecco's modified Eagle medium (DMEM), and antibiotics were supplied by Gibco (Life Sciences Inc., USA). All other chemicals and solvents were of analytical grade.

2.2. Plant collection, extraction, and determination of total polyphenol and flavonoids content

Fresh and healthy *L. vera* was collected from the suburbs of Kerman, Iran (30°16′60″N, 57°04′44″E) during August 2015. The plant identification was then performed by a specialist and it was deposited under accession number of 1422-3 in the herbarium of the department of pharmacognosy, faculty of pharmacy, Kerman university of medical sciences (Kerman, Iran). The plant leaves were thoroughly washed using double distilled water and kept for shade drying. After one week, the dried leaves were grinded into a fine powder in a mill and 5 g of the obtained powder were added to 100 mL of deionized water in a 250 mL Erlenmeyer flask. After 30 min heating at 80 °C, the extract was attained by centrifugation of the mixture (8000 rpm for 5 min) followed by keeping of the extract at 4 °C before being used for further investigations.

The total phenolic content (TPC) of the plant extract was estimated using the Folin-Ciocalteu procedure according to Baba and Malik [29]. The reaction mixture was prepared by adding 0.5 mL of herbal extract into 2 mL of distilled water and inserting 0.5 mL of F-C reagent, followed by thoroughly shaking of the mixture for 5 min. Afterwards, 2 mL of sodium carbonate solution (20% w/v) was added to the prepared reaction mixture and kept in the dark for further 60 min. The related absorbance of the sample was subsequently recorded at 650 nm using a UV–visible double beam PC scanning spectrophotometer (UV-1800, Shimadzu CO, USA) and the TPC was then calculated regarding to the calibration curve of gallic acid (mg of gallic acid per g dry weight). The total flavonoid content (TFC) of the herbal extract was assessed based on the method previously described by Elfalleh et al. [30]. Briefly, into 0.2 mL of the plant extract an amount of 1 mL methanol, 3.5 mL of distilled water, and 0.3 mL of $NaNO_2$ solution (5%) was added and the prepared mixture was incubated for 5 min. Thereafter, 0.3 mL of methanolic $AlCl_3$ solution (10%) was added to the reaction mixture and incubated for further 6 min, followed by inserting 1.7 mL of NaOH solution (1 M) and measuring of the related absorbance at 510 nm after 15 min incubation of the prepared mixture. The TFC was then calculated using the calibration curve obtained for the rutin as standard flavonoid and expressed as mg rutin per g dry weight.

2.3. Preparation and characterization of Zn NPs

An aqueous solution of $ZnSO_4$ (1 mM, 40 mL) was added to 10 mL of *L. vera* extract (as mentioned above) in a reaction vessel by passing pure argon in the solution. Subsequently, the reaction mixture was treated in a microwave oven (850 W, 60 s) to reduce the metal ions. The appearance of a dark gray color considered as a provisional marker for synthesis of Zn NPs. The produced nanostructures were then washed with chloroform, ethyl alcohol, and distilled water, respectively, and characterized using different techniques. The UV–visible spectrum of Zn NPs was recorded (200–600 nm) using the above mentioned spectrophotometer. In order to investigate the surface and elemental composition of the biogenic Zn NPs, samples were analyzed using a SEM apparatus (KYKY-EM3200) equipped with an energy dispersive X-ray (EDX) micro analyzer. The related size distribution pattern of NPs was plotted by manual counting of 400 individual particles from different SEM images. The crystalline structure of the prepared Zn NPs was examined by the X-ray diffractometer (Philips, PW1710) with CuKα radiation (λ = 1.5405 A°) in the 2θ range of 0°–80°. The surface chemistry of oven-dried (50 °C, 72 h) *L. vera* extract and Zn NPs was also investigated by a FTIR spectrophotometer (Shimadzu IR-470, Japan) at a resolution of 4 cm^{-1} in KBr disks.

2.4. Cell culture and cytotoxicity assay

Four human cancer cell lines including A549 (lung cancer), MCF-7 (breast cancer), HT-29 (colorectal cancer), and Caco-2 (colorectal cancer) were purchased from the Iranian Biological Resource Center (IBRC) Tehran, Iran. The cells were cultivated in DMEM medium supplemented with FBS (10%, v/v) and antibiotics [penicillin (100 U mL^{-1}) and streptomycin (100 μg mL^{-1}) at 37 °C in a CO_2 incubator (5% CO_2). In order to determine the cytotoxic effect of Zn NPs and $ZnSO_4$, the cells were harvested in the exponential phase of growth, seeded separately into 96-well tissue culture plates (10,000 cells per well) and allowed to adhere for 24 h followed by addition of the biogenic Zn NPs and $ZnSO_4$ into the desired wells to reach final concentration range of 10–160 μg mL^{-1} and