Reactive and Functional Polymers 120 (2017) 46–56

Contents lists available at ScienceDirect

Reactive and Functional Polymers

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

Preparation and characterization of cardamom extract-loaded gelatin nanoparticles as effective targeted drug delivery system to treat glioblastoma

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ARTICLE INFO

Keywords:
Cardamom
Gelatin
Glioblastoma
Drug delivery system
Cytotoxicity

ABSTRACT

The purpose of this research study was to prepare and characterize cardamom extract-loaded gelatin nanoparticles (CE-loaded GNPs) with a diameter ≤ 200 nm as a potent drug delivery system (DDS) for treatment of glioblastoma, which is the most common and aggressive type of brain tumor. The BBB poses physical and biological limitations to drug diffusion to reach target tissues. Polymeric nanoparticles, like gelatin, are suitable vehicles for drug delivery into the central nervous system (CNS). They are able to cross the BBB. Hence, we prepared CE-loaded GNPs by a two-step desolvation method. Seizures are detrimental secondary effects of brain tumors. Therefore, we used cardamom extract, which is an herbal anticancer and antiepileptic drug without any side effects, instead of synthetic drugs to load in gelatin nanoparticles during the particles preparation. We also prepared gelatin Type A and Type B nanoparticles for size comparison. Encapsulation efficiency, mean particle size, zeta potential and in vitro release profile were performed, and particle size analysis, dynamic light scattering (DLS), UV–Vis spectrophotometry, differential scanning calorimetry (DSC), X-Ray diffraction (XRD), scanning electron microscopy (SEM) and field emission scanning electron microscopy (FE-SEM) were employed to evaluate structural and physicochemical properties of the samples. CE-loaded GNPs were obtained with diameters of 40–200 nm, zeta potential of − 40.1 mV and entrapment efficiency (EE) of 70%. The ratio of extract to polymer, 1:20, was revealed to be more suitable in obtaining smaller nanoparticles without any precipitate or aggregation. We also examined cytotoxic effects of CE and CE-loaded GNPs on human glioblastoma cancer U87MG cells.

1. Introduction

Glioblastoma is one of the primary brain tumors that can destroy the brain tissues and cells very rapidly. Tumors produce symptoms mainly by mass effect and destruction of functioning brain tissue. Chemotherapy and radiation therapy cannot treat it effectively. Glioblastoma is a prevalent brain cancer, but common therapeutic efforts to target this aggressive brain tumor act prove to be futile due to several of the following factors: tumor cell resistance to conventional therapies, susceptibility of the brain to damage resulting from conventional therapies, limited capacity of neurons and the affected cerebral white matter for self-repair, inability of many drugs to cross the blood-brain barrier (BBB) to treat the tumor [1]. Various types of therapy have side effects. As mentioned, epilepsy is one of the most important secondary effects of brain tumors [2,3]. Thus, the patients, in addition to their medication side effects, must tolerate seizures and the side effects of antiepileptic drugs. This problem substantially increases patient fatigue.

The use of colloidal drug delivery systems (DDS) for diagnosis and treatment of brain and central nervous system (CNS) diseases and disorders is a great success for bioengineer. Kreuter [4] reported that by using polymeric nanoparticles with a diameter ≤ 200 nm, they could cross the blood-brain barrier (BBB) without obvious injury, and most peptides and macromolecules can cross the BBB as a result of this drug

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http://dx.doi.org/10.1016/j.reactfunctpolym.2017.09.008
Received 22 June 2017; Received in revised form 11 September 2017; Accepted 15 September 2017
Available online 18 September 2017
1381-5148/

delivery system. The size and shape of nanoparticles determines the kind of distribution and type of tissue pertinent for therapy [5,6]. In fact, polymeric nanoparticles, like gelatin nanoparticles (GNPs), with optimal surface modification and engineering are able to enter the brain to release the drugs. Thus, these nanocarriers protect the drugs from any damage until they reach the desired tissues and cells. Therefore, it is possible to employ this great ability of nanoparticles to diagnose and to treat brain and CNS diseases such as brain tumors and epilepsy [5,7–9].

Gelatin is a non-toxic natural polymer that has a unique chemical structure with a large number of useful amino acids and functional groups. It is a biocompatible and biodegradable polymer without harmful byproducts [10–15]. It can successfully undergo chemical modification, and by crosslinking, it can create novel opportunities for safe drug delivery nanocarrier synthesis and subsequent drug loading [16]. Thus, the gelatin nanoparticle is a safe nanocarrier to use in brain tissue that is sensitive to any damage and lacks self-repair capability.

The aqueous extract of cardamom gives rise to herbal anticancer and antiepileptic drugs without side effects. Cardamom (*Elettaria cardamomum* Maton), the queen of all flavors has a history as old as human race. It is one of the extravagant and fascinating flavors in the world. It is the dried fruit of an herbaceous perennial plant belonging to the ginger family, Zingiberaceae. The plant is indigenous to southern India and Sri Lanka. It is also cultivated in Guatemala. Cardamom has > 17 natural chemical structures that have many useful functional groups, like hydroxyl, carbonyl and amino acids, to increase levels of natural antioxidants, such as glutathione, in the blood and block radicals. They can also control and adjust the diffusion of ions to prevent seizures and inhibit excess ion release [17].

In this research study, we prepared the GNPs by a two-step desolvation method [16,18,19]. Then, we loaded aqueous CE during nanoparticle preparation to obtain CE-loaded GNPs to treat glioblastoma without side effects. We also evaluated the cytotoxic effects of CE and CE-loaded GNPs on human glioblastoma cancer U87MG cells.

2. Experimental procedure

2.1. Materials

Dried fruits of Indian green cardamom were purchased from a local market in Tehran (Iran). Gelatin Type A from porcine skin (300 Bloom) from Sigma-Aldrich (St. Louis, Mo, USA), Gelatin Type B from bovine skin (225 bloom) from Sigma-Aldrich (St. Louis, Mo, USA), Glutaraldehyde solution grade 1 (25% in water) from Sigma-Aldrich, Acetone from Sigma-Aldrich, Tween 80 (Polysorbate 80) 70% from Sigma-Aldrich, NaOH from Merck (Germany), and HCl from Merck (Germany) were purchased.

2.2. Preparation of aqueous extract of green cardamom

After cleaning the adulterant material, the fruits were ground with an electric grinder into a coarse powder. About 150 g of ground material was soaked in hot distilled water and placed in a shaking-incubator (GFL 3031) at 25 °C and 40 rpm, overnight. It was filtered through a cloth, and then, we put it in the shaking-incubator again at 25 °C and 40 rpm, overnight. We centrifuged (universal 320R hettich zentrifugen) the extract at 4500 rpm for 10 min to separate the large particles. Finally, we freeze-dried the extract, and the cream powder of the extract was obtained.

2.3. Preparation of cardamom extract-loaded gelatin nanoparticles (CE-loaded GNPs)

Gelatin nanoparticles were prepared by a two-step desolvation method. At first, 200–500 mg gelatin was dissolved in 10 ml distilled water, under conditions of heating at 40 ± 1 °C and magnetic stirring until a clear solution was obtained. After that, 10 ml acetone was added to the gelatin solution as a desolvating agent to precipitate the high molecular weight gelatin. The white supernatant was discarded, and then, the high molecular weight gelatin was redissolved by adding 10 ml of distilled water and subsequently stirring. The pH of the resulting solution was adjusted to between 9.2 and 9.4 by adding 0.1 M NaOH for gelatin Type A, but for gelatin Type B, the pH of the resulting solution was adjusted to < 4.8 by adding 0.1 M HCl. The aqueous extract of cardamom was added drop-by-drop with a syringe under magnetic stirring, followed by drop-wise addition of 30 ml acetone to form GNPs. Acetone was added until a permanent cream turbidity was obtained. Finally, 100 μL glutaraldehyde solution was added as a crosslinking agent, and the solution was stirred at 40 ± 1 °C for 30 min. Polysorbate 80 was added to coat the GNPs and allow them to cross the BBB, and the solution was then stirred. The solution was centrifuged at 15000 RPM for 30 min. The particles were purified by centrifugation and redispersion in water. Prepared nanoparticles were freeze-dried, thus cardamom extract-loaded GNPs were obtained.

The results of phytochemical analysis revealed that cardamom contains alkaloids, flavonoids, saponins, sterols and tannins. It is noticeable that the chemical structures, characteristics and pharmacological activities of saponins and flavonoids are so complex [17].

2.4. Characterization of gelatin nanoparticles

2.4.1. UV–Vis spectrophotometry

We determined the maximum wavelength that has the maximum absorption (λ max) for GNPs without extract and for cardamom extract.

2.4.2. Fourier transform infrared spectroscopy (FTIR)

By using the FTIR test, we can identify chemical compounds and chemical bonds in cardamom extract, gelatin nanoparticles and cardamom extract-loaded gelatin nanoparticles samples. It presents quantitative and qualitative analysis for the cardamom extract, GNPs and CE-loaded GNPs. We detected functional groups and characterized covalent bonding information in samples.

2.4.3. Particle size analysis

The particle size was determined by dynamic light scattering (DLS) using a Zetasizer Nano-ZS (Zeta sizer Nano ZS, Malvern Instruments). It also determined the polydispersity index (PDI).

2.4.4. Zeta potential (ZP)

Zeta potential values were measured using Laser Doppler Anemometry, employing a Zetasizer (Malvern Zetasizer Nano ZS).

2.4.5. Scanning electron microscopy (SEM)

The appearance, shape and size of nanoparticles were visualized by scanning electron microscopy. We also measured nanoparticle size.

2.4.6. Field emission scanning electron microscopy (FE-SEM)

In this technique, we employed very narrow probing beams with high electron energy. Therefore, this method protects susceptible nanoparticles like GNPs against damage, disintegration and charging due to high energy and temperature, and we obtained images with high spatial resolution.

2.4.7. Entrapment efficiency (EE)

The total quantity of CE contained in the GNPs was determined by a 'direct' technique. 5 mg of the nanoparticles were added to 5 ml PBS. After complete dissolution, the drug concentration was determined by UV–Vis spectrophotometer at λ = 302 nm.

$$EE\% = \frac{\text{Total of entrapped extract in GNPs}^*}{\text{Total of extrat}}\ 100 \quad (1)$$