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Synthesis and antidiabetic activity of selenium nanoparticles in the presence of polysaccharides from *Catathelasma ventricosum*

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ABSTRACT

Selenium nanoparticles (SeNPs) were prepared by adding *Catathelasma ventricosum* polysaccharides (CVPs) to the redox system of selenite and ascorbic acid. Taking particle size as an investigation index, the optimal synthesis conditions of CVPs-SeNPs were obtained by orthogonal test. Herein, the diameter, morphology, and stability of the CVPs-SeNPs were characterized by dynamic light scattering (DLS) and transmission electron microscopy (TEM). Moreover, the antidiabetic activities of CVPs-SeNPs were evaluated by STZ (streptozocin)-induced diabetic mice. The obtained results showed that, optimum synthesis conditions of CVPs-SeNPs were: ultrasonic time 60 min, concentration of Vc 0.04 M, reaction time 2 h, pH 7.0. Under these conditions, mean diameter of the synthesized CVPs-SeNPs was around 49.73 nm. TEM of CVPs-SeNPs prepared in optimal conditions showed individual and spherical nanostructure. CVPs-SeNPs (particle size of about 50 nm) could be stable for approximately 3 months at 4 °C, but only 1 month at 25 °C. The results on serum profiles and antioxidant enzymes levels revealed that CVPs-SeNPs had a potential antidiabetic effect. In addition, CVPs-SeNPs showed significantly higher antidiabetic activity ($p < 0.05$) than other selenium preparations such as SeNPs, selenocysteine, sodium selenite.

1. Introduction

Diabetes mellitus (DM) is a highly prevalent disease that describes a group of metabolic disorders characterized by hyperglycemia. It affects about 415 million people aged 20–79 years in the year 2015, and this number is predicted to rise to 642 million by 2040 [1]. Prolonged hyperglycemia leads to the production of free radicals and reactive oxygen species (ROS), resulting in the increase of level of oxidative stress [2]. Nevertheless, it has been reported that excessive ROS can directly reduce the activities of anti-oxidative enzymes and destroy membrane lipids, which initiates and promotes the development of DM. In fact, the treatment with appropriate antioxidant has been showed to significantly reduce and repair the oxidative damage in patients with diabetes by scavenging ROS and mitigating oxidative stress. Besides, hyperlipidemia is related to the development of variety diabetes complications, hence is the major cause of morbidity and mortality of diabetes as well. However, so far, treatment of diabetes by insulin and oral hypoglycemic drugs fails to prevent diabetes related complications in many patients [3]. Above all, we can speculate that the key to the prevent and treatment of diabetes and its complications is to find a novel, efficient and cost effective agent with numerous bioactivities, including antihyperglycemia, antioxidant and antihyperlipidemia.

Selenium (Se) is an essential trace element for human body, it has been confirmed that selenium treatment can be efficient in eliminating diabetes-induced structural alterations [4]. Furthermore, a majority of research studied on antioxidants and several studies reported that selenium administration improve antioxidant levels and protects the body from DM [5,6]. For instance, Se has been reported to be effective in protecting oxidative damage of the liver, kidney, and heart [7]. Nevertheless, the bioavailability and biological activities of selenium are extremely restrained on account of the narrow margin between the thresholds of functionality and toxicity [8]. There is abundant evidence that the bioavailability and toxicity of Se are related to its different chemical species. Recently, selenium nanoparticles (SeNPs), a sort of red elemental selenium (Se^0) in colloidal state, are attracting more and more attention due to their excellent high bioavailability, biological activity and low toxicity. Previous study has shown that biologically synthesized selenium nanoparticles with diameter <100 nm have potential application as food additives with antioxidant properties [8]. In addition, as for toxicity, SeNPs has a 7-fold lower acute toxicity than

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sodium selenite, a 3-fold lower acute toxicity than organic selenium in mice (LD_{50} 113, 15, 30–40 mg Se/kg body weight, respectively) [9]. What's more, as a kind of nano material, SeNPs also have nanometer size effect, which means the smaller nanoparticles they are, the higher activities they have. Studies have indicated that SeNPs had nanometer size effect in antioxidant activity as well. However, SeNPs are very unstable in liquid phase and are extremely easy to aggregate and form gray or black selenium with large particle size, thus, the bioavailability and activities of SeNPs will be lost. So, it is important to maintain the good stability of SeNPs in liquid phase. One of the effective methods is to add stabilizing agents, such as polysaccharides, proteins, surfactants and so on [10].

Polysaccharides have complex branch structures and active hydroxyl groups so that it can modify the interface of nanoparticles, control the growth of nanoparticles and stabilize the nanoparticles solution. In addition, many polysaccharides have been proved to possess varying biological activities, so it can be expected to generate synergistic effects between polysaccharides and SeNPs. *Catathelasma ventricosum*, an edible mushroom from southwest China, has a pleasant taste and a variety of medicinal activities. In previous study, we have found that the polysaccharides extracted from *Catathelasma ventricosum* (CVPs) possess potent antioxidant activity and have much protective effects on liver, kidney and pancreas tissues. Moreover, the study also indicated that CVPs are safe for normal mice [11]. However, to the best of our knowledge, no or few studies have reported the preparation and stabilization of SeNPs by using CVPs as a disperser and stabilizer in an aqueous solution.

To date, three different approaches can be used for synthesis of SeNPs covering the physical, chemical, and biological techniques. There are lots of successfully applied methods such as chemical reduction [12], microwave synthesis [13], biosynthesis [14], and so forth. In current study, we prepared a kind of SeNPs stabilized by CVPs (CVPs-SeNPs), likewise, the optimal preparation conditions and antidiabetic activities (including antioxidant, antihyperglycemic and antihyperlipidemic activities) of CVPs-SeNPs were obtained. Besides, we also assessed the antidiabetic activities of other selenium preparations, including SeNPs, selenocysteine (organ selenium) and sodium selenite (inorganic selenium), in order to further evaluated the antidiabetic activity level of CVPs-SeNPs. Of note, there were some studies focused on the combination effect of SeNPs and vitamin E (V_E, a kind of antioxidant agents) on various respects, and the results have showed that the combination of V_E and SeNPs had positive and effective effects [15,16]. Thus, we anticipated that the combination of V_E and CVPs-SeNPs might be effective in amelioration of STZ-induced diabetes, the antidiabetic activities of V_E, CVPs-SeNPs, and their combination in STZ-induced diabetes mice were evaluated in this study as well. In a word, the main purpose of this study is to optimize the preparation conditions and assess the antidiabetic activity of CVPs-SeNPs, and more predominantly, provide a theoretical basis for the development of novel antidiabetic agents.

2. Materials and methods

2.1. Chemicals

Samples of *Catathelasma ventricosum* were purchased from the Mianyang Edible Fungi Research Institute (China) and maintained using synthetic potato dextrose agar (PDA) medium. Sodium selenite (Na_2SeO_3, ≥97%, Chemical grade) was purchased from Shandong West Chemical Industry Co., Ltd. (China). Ascorbic acid (Vc), Vitamin E (V_E) and metformin hydrochloride were purchased from Sangon Biotech Co., Ltd. (Shanghai, China), sodium hydroxide (NaOH) and acetic acid (HAc) were acquired from Chengdu Kelong Chemical Reagent Plant (China). Selenocysteine (HPLC ≥ 98%) was acquired from Yuanye Biological Technology Co., Ltd. (Shanghai, China). Glibenclamide, streptozocin (STZ) were purchased from Sigma-Aldrich Co. LLC. (USA). Accu-Chek Performa glucometer and blood glucose test strips were purchased from Beijing Boai Harbour Trading Co., Ltd. (China). Reagent kits for the determination of insulin, total cholesterol (TC), triglyceride (TG), low density lipoproteincholesterol (LDL-C), high density lipoproteincholesterol (HDL-C), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) were obtained from the Jianchen Bioengineering Institute (China). The water used in the experiment was ultrapure water (electrical resistivity 18.2 MΩ·cm), additionally, all other chemicals and solvents were of analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (China).

2.2. Synthesis of CVPs-SeNPs

2.2.1. Synthetic method of CVPs-SeNPs

CVPs-SeNPs were synthesized by a modified process [17]. The methods of extraction and purification of CVPs referred to Liu et al. [18]. In a typical procedure for the synthesis of CVPs-SeNPs, CVPs solution (1 mg/ml, 5 ml) was mixed with 5 ml 0.01 M sodium selenite under magnetic stirring at 25 °C, and then, treated the mixture with ultrasonic during some time. Next, 5 ml freshly prepared ascorbic acid solution was slowly added into the mixture, and the pH of reaction system was immediately adjusted using NaOH and HAc. After a certain time of reaction, the residual Na_2SeO_3 was removed by dialysis against Milli-Q water at 4 °C, when no Se was detected in the outer solutions as determined by inductively coupled plasma-mass spectrometry (ICP-MS, Perkin Elmer Nexion 300, USA) analysis, meant the liquid phase of CVPs-SeNPs was prepared eventually. Furthermore, the way to synthesis SeNPs was the same to above method, but there was a need to replace aqueous solution of CVPs with the equal volume of Milli-Q water.

2.2.2. Optimization of synthesis conditions of CVPs-SeNPs

In our preliminary experiment, we discussed the effects of reaction temperature, reaction time, pH, concentrations of Vc, CVPs and ultrasonic time on the particle size of CVPs-SeNPs by single-factor test. The results showed that ultrasonic time, Vc concentration, reaction time and pH had significant impacts on the particle size of CVPs-SeNPs compared with other factors. Thus, in this work, the optimum synthesis conditions of CVPs-SeNPs were investigated by orthogonal test. The selected factors and levels were shown in Table 1.

2.3. Characterization and measurements

The morphology of the SeNPs and CVPs-SeNPs were observed using TEM (Zeiss Libra 200FE, Germany). For TEM observation, the samples need to be dispersed to the copper line for drying after ultrasonic treatment. The mean diameter was determined by DLS analysis using Nano ZS instrument (Nano-Zs 90, UK).

2.4. Stability of CVPs-SeNPs

The stability of CVPs-SeNPs was determined by measuring diameters of the CVPs-SeNPs stored in 4 °C and 25 °C for 0, 20, 40, 60, 80, 100, 120 days, respectively.

Table 1
Four factors and their corresponding levels in the study.

Levels	**Factors**			
	A Ultrasonic time (min)	B Concentration of Vc (M)	C Reaction time (h)	D pH
1	55	0.03	2	6.5
2	60	0.04	3	7.0
3	65	0.05	4	7.5