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Preparation and physicochemical properties of chitosan broadleaf holly leaf nanoparticles

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ABSTRACT

Objective: To prepare chitosan broadleaf holly leaf nanoparticles and to investigate their physicochemical properties and drug release characteristics in vitro.
Methods: Broadleaf holly leaf total flavonoids were extracted using an ethanol gradient and the determination of their contents was performed using a UV–vis spectrophotometer. Nanoparticles were prepared by ionic crosslinking. The morphology and distribution of the particles were examined. In addition, several factors affecting drug-loading and the encapsulation rate were explored. Furthermore, studies on the characteristics of in vitro release were performed.
Results: The content of total flavonoids from broadleaf holly leaf was 31.84%. The nanoparticles were spherical in shape, and the size range was between 100 and 600 nm. The polydispersity was 0.137. The optimal preparation process of the nanoparticles was the following: chitosan concentration 1 mg/ml, TPP concentration 1 mg/ml, and concentration of broadleaf holly leaf total flavonoids l mg/ml. The in vitro release profile showed that the nanoparticles have an initial burst release effect of 45.6–48.9% within the first two hours. The total release was 91.9% in 24 h.
Conclusions: Chitosan nanoparticles are a potential broadleaf holly leaf delivery system.

1. Introduction

Hyperlipidaemia is an independent risk factor for coronary heart disease, stroke and other cardiovascular and cerebrovascular diseases (Skoumas et al., 2013). In addition, it is closely related to obesity, fatty liver, hypertension, high blood sugar and other aging diseases (Smith and Singleton, 2013). According to the Center for Disease Control, a survey of 1492 physicians who provide ambulatory care in non-government settings revealed that hyperlipidaemia was second only to hypertension on the list of the 10 most common chronic conditions (Nelson, 2013). Thus, prevention and treatment of hyperlipidaemia plays an essential role in the prevention of coronary heart disease, stroke and other diseases.

At present, chemical drugs for the treatment of hyperlipidaemia include mainly statins and fibrates (Zsiros et al., 2014) which have specific side effects. Statin drugs have adverse reactions, such as effects on the gastrointestinal tract (Geng et al., 2014) and liver damage (Anon., 2004; Heuer et al., 2000), and the most serious adverse reaction is rhabdomyolysis (Catapano, 2012) in which the degree of danger increases greatly, particularly when it is used in combination with fibrates. Thus, it is particularly important to identify drugs with effects of decreasing TC and TG and whose adverse reactions are weak.

Natural medicines, particularly traditional Chinese herbal medicines, which are safe and have little side-effect, have gained attention in lipid-lowering drug research (Lee et al., 2013; Liu et al., 2013). Broadleaf holly leaf (broadleaf holly leaf C.J. Tseng) is a common plant in Guangxi and has been characterized by Guangxi Zhuang Nationality Drug Quality Standards. Previous studies have indicated that broadleaf holly leaf has detoxification, bactericidal anti-inflammation, and antioxidation effects and its effects on reducing lipids have been reported in numerous studies (Li et al., 2013). The mechanism of broadleaf holly leaf in reducing lipids might be via the inhibition of ACAT (acyl CoA cholesteryl acyl transferase), an enzyme that catalyses the intracellular esterification of cholesterol in various tissues (Li et al., 2013). However, due to the bitter taste of broadleaf holly leaf, its compliance is poor. In addition, when it is used to reduce lipids, the required dosage of broadleaf holly leaf is relatively large, which induces acute renal damage. Thus, we aimed to improve the compliance and to reduce its quantity to achieve a better lipid-lowering effect.

Nanotechnology has been an emerging research direction in recent decades in the medical field. Due to the remarkable

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properties of nanoparticles, they are widely used in the fields of medicine, materials and life sciences, and other areas of study (An and Jin, 2012; Giannitrapani et al., 2014). Moreover, the smaller the particle size, the greater the surface area of the nanoparticles. Furthermore, nanoparticles demonstrate passive targeting to the liver and thus can be phagocytosed by the reticuloendothelial system (RES) which is distributed in the liver. Consequently, this can greatly increase the bioavailability of the drug. In addition, nanoparticles can also conceal the bad taste of a drug. Thus, nanoparticles of broadleaf holly leaf can resolve some of the current existing problems of renal damage and poor compliance.

The aim of this study was to prepare the nanoparticles of broadleaf holly leaf via ionic crosslinking and to investigate the impact factors of drug-loading and encapsulation. Furthermore, release in vitro was investigated to determine the efficacy of this system.

2. Materials and methods

2.1. Materials

Chitosan (degree of deacetylation 90.2%, molecular weight 21 kDa) was made from a shrimp shell and obtained from Shandong Aokang Biotech Ltd. (Shandong, China). Broadleaf holly leaf was provided by Huang's Broadleaf Holly Leaf Company (Daxin County, Guangxi Zhuang Autonomous Region, China). It was identified as one of the species in the *Ilex* genus by Wang Jie (senior technician of Guangxi Medical University). Rutin was purchased from the China National Institute for Food and Drug Control (batch number 10080-200306; content >98%). All other reagents used in this study were of analytical grade.

2.2. Methods

2.2.1. Extract of total flavonoids in broadleaf holly leaf

Samples of broadleaf holly leaf were pulverized into coarse powder and digested by 95%, 75%, 50% and 20% ethanol 10 times for 2 days. After filtration, all of the filtrate was combined and concentrated, and crude products were obtained. Next, distilled water was added to the sample and evenly mixed to remove any impurities, including resin and lipid-soluble pigments. Chitosan was then used as flocculants to purify the intermediates, which was filtered and centrifuged at high speed to obtain the supernatant solution. Finally, total flavonoid extracts of broadleaf holly leaf were obtained by freeze-drying. Fig. 1 shows these several steps in a flow chart.

2.2.2. Determination of total flavonoid extracts of broadleaf holly leaf

2.2.2.1. Determination of the detected wavelength. Totalflavonoid extracts of broadleaf holly leaf and a rutin reference substance were placed in a 10-ml volumetric flask. Next, 0.3 ml of a 5% sodium nitrite solution was added, shaken and incubated for 6 min. A mixture of 0.3 ml of a 10% aluminium nitrate solution was then added, shaken and incubated for 6 min, followed by 4 ml of a 1 mol/l sodium hydroxide solution and 30% ethanol. The solution was shaken and incubated for 10 min. The maximum wavelength of the UV–vis absorption was determined using full wave scanning.

2.2.2.2. Preparation of the rutin standard curve. Calibration curves for the rutin standard solutions were prepared at concentrations of 10, 20, 30, 40, 50, and 60 mg/L. The absorbance was measured using methods discussed in Section 2.2.2.1.

2.2.2.3. Content determination of the total flavonoids of the broadleaf holly leaf. The measurement methods were described as the following: 3 g of broadleaf holly leaf extract was obtained with accurate weight and placed into a 100-ml flask with 30% ethanol. The solution was dissolved by ultrasound, and 30% ethanol was added to the mark. Precisely 1.00 ml of filtered liquid was obtained after the solution was mixed and filtered, and the sample was subsequently placed into a 100-ml volumetric flask. Next, 30% ethanol was added to the mark and the mixture was shaken. Subsequently, 4.00 ml solution was obtained into a 10-ml volumetric flask and the absorbance was measured according to Section 2.2.2.1.

2.2.3. Preparation of chitosan broadleaf holly leaf nanoparticles

Chitosan broadleaf holly leaf nanoparticles were prepared using the following methods, which have been previously described (Zhang et al., 2011, 2012). Briefly, Chitosan was dissolved in 1% acetic acid to acquire chitosan solutions at different concentrations. Next, tripolyphosphate (TPP) was dissolved in distilled water at different concentrations. A specific volume of chitosan solution was obtained and slowly added into the total flavonoids of the broadleaf holly leaf solution at room temperature with magnetic stirring (600 rpm). This solution was slowly placed into the TPP solution using a 4th syringe needle for 45 min until a blue opalescence appeared (Elsayed et al., 2011; Sonaje et al., 2010). Finally, the chitosan broadleaf holly leaf nanoparticles were obtained.

2.2.4. Characterization of chitosan broadleaf holly leaf nanoparticles

Particle size and distribution are the main characteristics of the nanoparticles. They were obtained using a particle sizer (Zetasizer 3000 HAS, Malvern Instruments Ltd., Worcs, UK). The morphology of the nanoparticles was examined using scanning electron microscopy (TEM) (H-7650, Japan Hitachi).

2.2.5. Determination of entrapment efficiency

Entrapment efficiency (EE) is the percentage of the drug that is entrapped in nanoparticles of the colloidal solution and represents the effects of total flavonoid extracts, chitosan and TPP on the entrapment efficiency. The nanoparticles were centrifuged at 15000 rpm and 4 °C for 30 min, and the amount of free flavonoids in clear supernatant was determined using UV–vis spectrophotometry (Shimadzu UV-1601, Shimadzu Co., Ltd., Japan).

$$\mathrm{EE}(\%) = \left[\frac{(\text{Total flavonoids in broadleaf holly leaf} - \text{the amount of flavonoids in broadleaf holly leaf in supernatant})}{\text{Total flavonoids in broadleaf holly leaf}}\right] \times 100\%$$

The calculation formula is as follows:

2.2.6. Determination of drug loading

Drug loading (DL) refers to the weight percentage contained in particulate drug formulations which is an important standard used to evaluate the quality of the microparticles. The fixed