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Research paper

Enhancement of anti-inflammatory activity of glycyrrhizic acid by encapsulation in chitosan-katira gum nanoparticles

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ABSTRACT

Efforts were made to improve the bioavailability and efficacy of Glycyrrhizic acid, a triterpentine saponin obtained from *Glycyrrhiza glabra*, having several pharmacological properties, by its encapsulation in biocompatible biopolymeric nanoparticles. Polycationic chitosan and polyanionic gum katira were used to prepare nanoparticles by ionic complexation method. Glycyrrhizic acid was loaded into the nanoparticles and was then examined for change in its *in vivo* anti-inflammatory activity against carrageenan-induced rat hind paw inflammation. The effects of concentrations of glycyrrhizic acid, chitosan and katira gum, upon particle size and encapsulation efficiency of glycyrrhizic acid were studied with the help of response surface methodology employing 3-factor, 3-level central composite experimental design. Particle size and encapsulation efficiency of optimized nanoparticulate formulation were 175.8 nm and 84.77%, respectively. Particles were observed in transmission electron microscopy to be spherical in shape and 80 nm in size. FTIR analysis indicated electrostatic interactions between carboxyl groups of ammonium glycyrrhizinate and amino groups of chitosan. *In vitro* drug release studies indicated that glycyrrhizic acid was released from the nanoparticles following zero-order kinetics and that there was a sustained release of the drug with 90.71% of it being released over a 12 h period, and that the mechanism of release of glycyrrhizic acid from the nanoparticles was a combination of diffusion and erosion of the polymer matrix. *In-vivo* anti inflammatory efficacy of glycyrrhizic acid clearly improved upon encapsulation in chitosan-katira gum nanoparticles, by overcoming the limited bioavailability of its other forms.

1. Introduction

Glycyrrhizic acid is a triterpentine saponin obtained from *Glycyrrhiza glabra* having several pharmacological properties including anti-inflammatory, antiulcer, anti-allergic, immune-modulating, and antiviral properties [1–5]. Its anti-inflammatory activity is moderate as its potency is less than the steroidal or non-steroidal anti-inflammatory drugs such as prednisolone, dexamethasone, indomethacin and diclofenac [6]. Therapeutic potential of glycyrrhizic acid is limited due to its poor solubility in water as well as in biological fluids resulting in its low bioavailability [7]. While glycyrrhetic acid appears in the blood circulation after oral administration of glycyrrhizin [8,9], pharmacokinetic studies have shown that oral absorption of glycyrrhetic acid (or its salt) is extremely ineffective. The *i.v.* administration with a high dose of glycyrrhizin shows side effects of the steroidal drugs, such as salt retention and hypokalemia [10,11]. In order to overcome this problem, various glycyrrhizic acid preparations have been researched in recent years. These include glycyrrhizic acid liposome [12], glycyrrhizic acid-chitosan nanoparticles delivery system [13], and cyclodextrin inclusion complexes [14].

From a biopharmaceutical point of view, chitosan can act as an absorption enhancer across intestinal epithelial because of its mucoadhesive and permeability enhancing property [15]. It breaks down to harmless products (amino sugars), that are completely absorbed by the human body; therefore, it is biocompatible with living tissues [16]. It has been used to improve the dissolution rate of the poorly soluble drugs [17–19], drug targeting [20,21] and reported to have anti-inflammatory potential [22] as well, thereby giving an indication of it being an effective candidate for delivery of poorly soluble drugs. It is composed of 2-amino-2-deoxy-h-D-glucan combined with glycosidic linkages [23] and its polycationic nature allows ionic cross linking of its free amine groups with multivalent ions [24]. Nanoparticles of chitosan can be prepared by the very common method of ionic complexation and being an

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easy to scale up process it can be utilized on commercial scale also. The present work involves formation of nanoparticles by ionic cross linking between the positively charged amino groups of chitosan and the negatively charged groups of gum katira. Katira gum is a non-toxic, anionic natural polysaccharide isolated from *Cochlospermum religiosum* [25] which has been recently reported as novel suitable pharmaceutical excipient [26]. It has also been proposed as a suitable carrier for colon targeting as it is resistant to acidic pH in stomach but is susceptible to degradation by colonic bacterial enzymes [27,28]. Nanoparticulate delivery system composed of chitosan-katira gum may not only control the rate of drug release but also improve its oral bioavailability and enhance its absorption. Till date very few studies have been conducted on entrapment of glycyrrhizic acid in polymeric nanoparticles to improve its anti-inflammatory potency. In the present work glycyrrhizic acid-loaded polymeric nanoparticles were prepared and evaluated for its anti-inflammatory potential using carrageenan induced rat paw edema method.

2. Materials and methods

2.1. Materials

Glycyrrhizic acid was procured from Sigma Aldrich (St. Louis, USA). Katira gum was procured from local shop (Hisar, India). Pluronic F-68 and carrageenan were purchased from Hi-Media Laboratories Pvt. Ltd. (Mumbai, India). All other chemicals used were of analytical grade. Wistar rats, 100–200 g each, were purchased from disease free small animal house, Lala Lajpat Rai University of Veterinary and Animal Sciences (Hisar, India) [Regn. No. 1669/GO/abc/12/CPCSEA/dated 08-4-2013] after approval of Institutional Animal Ethics Committee, Guru Jambheshwar University of Science & Technology, Hisar (Approved by CPCSEA, Govt of India, Regn. No CPCSEA/0436).

2.2. Preparation of glycyrrhizic acid-loaded chitosan-katira gum nanoparticles

Glycyrrhizic acid loaded chitosan-katira gum nanoparticles were prepared by ionic complexation method using Pluronic-F68 as a stabilizer. Briefly, an aqueous solution of katira gum (0.005–0.01%, w/v) containing Pluronic F-68 (1%, w/v) and glycyrrhizic acid (25–50%, w/w of polymer) was prepared. To this, chitosan solution (0.005–0.03%, w/v) was added under sonication for 15 min to prepare ionically crosslinked nanoparticles. Particle size analysis was carried out with the nanosuspension so obtained. Optimized formulation was freeze-dried for 24 h at −90 °C at 0.0010 mbar using laboratory model freeze-dryer (Alpha 2-4 LD Plus, Martin Christ, Germany).

2.3. Experimental design

Preliminary trials were carried out to select the optimum formulation variables and ingredient concentrations. Design Expert Software (Version 8.0.4, Stat-Ease Inc., Minneapolis, MN) was used to design experiments, and for carrying out statistical analysis. Optimization of glycyrrhizic acid loaded chitosan-katira gum nanoparticles was carried out using a central composite design with $\alpha = 1$ as per standard protocol. Three factors i.e. concentrations of katira gum, glycyrrhizic acid and chitosan were varied and factor levels were suitably coded. Stabilizer concentration and sonication time were kept constant. Particle size and encapsulation efficiency were chosen as the response variables.

2.4. Characterization of glycyrrhizic acid-loaded katira gum nanoparticles

2.4.1. Particle size

Particle size of nanosuspension was analyzed using dynamic light scattering technique. One milliliter of nanosuspension was scanned in disposable sizing cuvette at 25 °C in particle size analyzer (Zetasizer Nano ZS90, Malvern, UK).

2.4.2. Zeta potential

Zeta potential of glycyrrhizic acid nanoparticles was analyzed at 25 °C. One milliliter of the nanosuspension was loaded in clear disposable zeta cell and scanned with an equilibration time of 120 s (Zetasizer Nano ZS90, Malvern, UK).

2.4.3. Encapsulation efficiency

For the determination of encapsulation efficiency, nanosuspension was centrifuged (Cooling centrifuge, 4K-15, Sigma, Germany) at 10,000 rpm for 45 min at 4 °C. Supernatant was analyzed spectrophotometrically (UV spectrophotometer 2450, Shimadzu) for free glycyrrhizic acid at 256.5 nm. Encapsulation efficiency (%) was calculated as follows:

$$\%\ Encapsulation\ efficiency = \frac{(G_t - G_f)}{(G_t)} \times 100 \quad (1)$$

where G_t – total glycyrrhizic acid, G_f – free glycyrrhizic acid.

2.4.4. Morphology

Morphology of optimized glycyrrhizic acid-loaded chitosan-katira gum nanoparticles was observed by Transmission Electron Microscopy (TEM). A drop of nanosuspension was loaded onto a copper grid, air-dried and observed. TEM micrograph was captured at 80 kV accelerating voltage under 60,000× magnification factor.

2.4.5. Fourier-transform infrared spectrophotometer (FTIR)

Glycyrrhizic acid, lyophilized powders of optimized batches of blank chitosan-katira gum and glycyrrhizic acid-loaded chitosan-katira gum nanoparticles were analyzed using Fourier transform infrared spectrophotometer (IR Affinity-1, Shimadzu, Japan) in the range of 4500–500 cm^{-1} as KBr pellets.

2.5. In-vitro release studies

In vitro release of glycyrrhizic acid from optimized batch of glycyrrhizic acid-loaded chitosan-katira gum nanoparticles was carried out using dialysis sac method employing USP type-II dissolution rate apparatus (TDT-08L, Electrolab, Mumbai, India). Accurately weighed 500 mg of lyophilized glycyrrhizic acid loaded chitosan-katira gum nanoparticles was dispersed in 5 ml of phosphate buffer saline (pH 7.4) and placed in a dialysis sac. Dialysis sac was then immersed in 100 ml of phosphate buffer saline with the help of sinkers. The release medium was maintained at 37 ± 0.5 °C and rotated at 100 rpm. Sample aliquots of 3 ml were withdrawn at various time intervals up to 12 h and analyzed for glycyrrhizic acid contents by measuring absorbance at 256.5 nm (UV spectrophotometer 2450, Shimadzu).

2.6. In-vivo anti-inflammatory studies

Carrageenan induced paw inflammation model was used to study anti-inflammatory activity in Wistar rats as earlier reported [29]. The protocol of the study was approved by the Institutional Animal Ethics Committee (Regn. No CPCSEA/0436) vide. Endst. No. IAEC/174-182, dated 19th Dec., 2013. Rats were divided into 7 groups of 6 animals per cage and treated orally with saline water