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Evaluation of anti-diabetic activity of glycyrrhizin-loaded nanoparticles in nicotinamide-streptozotocin-induced diabetic rats

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ABSTRACT

Glycyrrhizin is an active constituent of the roots and rhizomes of *Glycyrrhiza glabra* and has anti-hyperglycemic effects. In this study, nanoparticles (NPs) loaded with glycyrrhizin or metformin were evaluated *in vivo* for their anti-hyperglycemic potency towards type-II diabetes in rats. The NPs were produced *via* the ionotropic gelation method using the biocompatible polymers chitosan and gum arabic. The polymer concentration was optimized using the 3^2 factorial method to acquire both minimum particle size and maximum encapsulation efficiency. The NPs were then characterized with respect to particle size, encapsulation efficiency, stability, chemical interactions, and *in vitro* drug dissolution profiles using spectroscopic and microscopic analysis. Furthermore, glycyrrhizin and metformin and their nanoformulations were administered for 21 successive days to diabetic rats. Glycyrrhizin-loaded NPs had significant anti-diabetic effects even though they contained approximately one quarter of the dosage relative to the pure form.

1. Introduction

Diabetes is a group of metabolic disorders affecting the metabolism of carbohydrates, fats, and proteins. It commonly impairs glucose homeostasis because either the body (*i.e.*, beta cells in the pancreas) does not produce enough insulin (type I diabetes) or the cells do not respond to endogenously-produced insulin (type II diabetes) (James, 2005). According to a recent report from the World Health Organization (2016), diabetes is the fastest growing chronic disease in the world; the number of diabetic patients is increasing particularly in the world's middle-income countries where the rate increased from 4.7 to 8.5% between 1980 and 2014. Almost half of all deaths before the age of 70 have been attributed to high blood glucose levels associated with such diseases (World Health Organization, 2016). Herbal products have attracted more attention than synthetic drugs for the treatment of human disease due to their safety profiles and price (Wachtel-Galor and Benzie, 2011).

Glycyrrhiza glabra (from the family Fabaceae), also known as mulethi, has been recognized for a multitude of advantageous pharmacological properties such as antitussive and expectorant, anti-bacterial, anti-microbial, anti-fungal, anti-inflammatory, anti-malarial, nootropic, anti-depressant, anti-convulsant, anti-oxidant, hepatoprotective, anti-HIV, anti-viral, anti-tumor, immunomodulatory, anti-dyslipidemic, and anti-hyperglycemic effects (Asl and Hosseinzadeh, 2008; Roshan et al., 2012; Damle, 2014; Kalsi et al., 2016). Glycyrrhizin is a triterpenoid saponin and the major bioactive component extracted from the roots and rhizomes of licorice (*G. glabra*), which possesses a sweet taste. Glycyrrhizin is made up of two molecules of D-glucuronic acid and one molecule of 18β-glycyrrhetinic acid, which is the principal aglycone formed after the hydrolysis of glycyrrhizin, and is absorbed by the blood (Shams et al., 2015). Glycyrrhizin has also been reported to possess pharmacological activities such as anti-bacterial (Krausse et al., 2004), anti-inflammatory (Wang et al., 2016), anti-malarial (Kalani et al., 2013), anti-depressant (Dhingra and Sharma, 2005), anti-oxidant (Imai et al., 2013), hepatoprotective (E-Magd et al., 2015), HIV-replication inhibiting (Sasaki et al., 2002), anti-viral (Duan et al., 2015), anti-tumor (Huang et al., 2014; Roohbakhsh et al., 2016), immunomodulatory (Tu et al., 2012), anti-allergen (Li and Zhou, 2012), anti-thrombotic (Paula et al., 2013), and cardioprotective effects (Parisella et al., 2012). In addition to these pharmacological activities, glycyrrhizin also has hypolipidemic and anti-hyperglycemic activities (Takii et al., 2001; Kalaiarasi et al., 2009; Kalaiarasi and Pugalendi, 2009; Sen et al., 2011).

Nanoparticulate drug delivery systems have been used to modify

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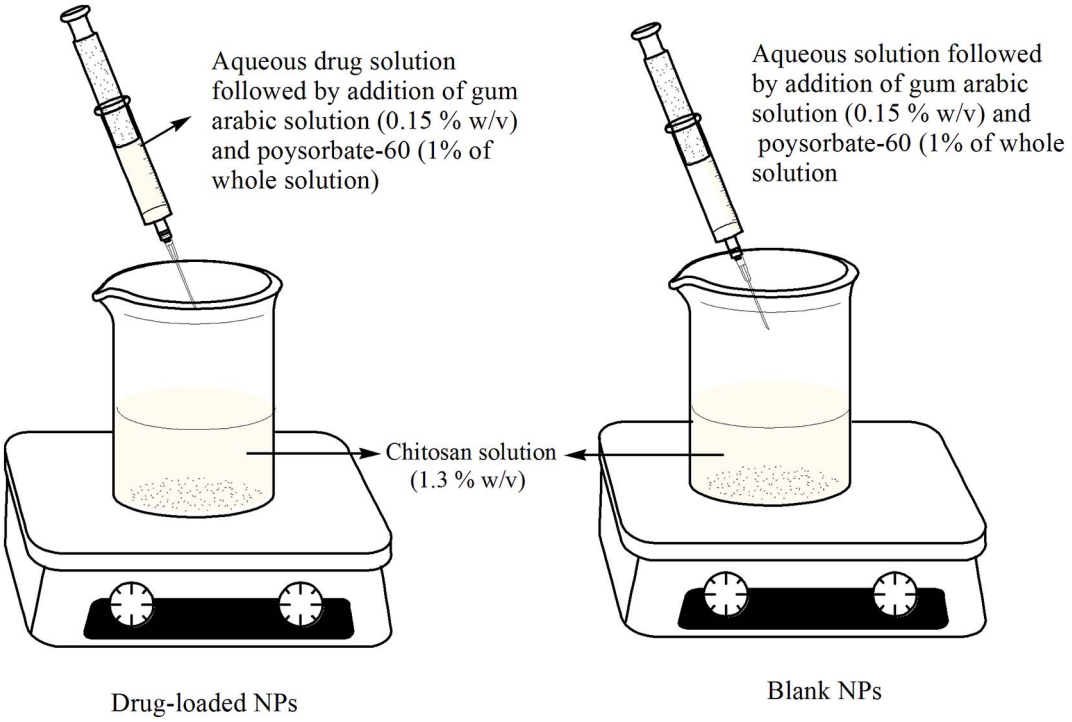

Fig. 1. Schematic of the synthesis of drug-loaded NPs and blank NPs.

and enhance pharmacokinetic properties, such as absorption, distribution, metabolism, and excretion, of various drugs used in therapeutic applications (Singh et al., 2011). Due to the poor bioavailability of certain drugs, they are not efficiently absorbed and/or distributed in the body. Hence, those drugs may not attain a high enough systemic concentration to exert their pharmacological effects. Glycyrrhizin as well as metformin, both have been reported for their poor/low bioavailability after oral administration due to slow and incomplete absorption in the gastrointestinal tract (Jin et al., 2012; Mirazi et al., 2015). Nanoparticulate systems offer great promise in the conversion of poorly-soluble and/or poorly-absorbed bioactive compounds into efficiently-deliverable drugs (Saraswathi et al., 2014). Nanoformulations therefore have advantages over conventional formulations in many respects such as enhanced solubility and bioavailability, targeted drug delivery, sustained drug release, dose reduction, and the minimizing of possible side effects (Kumar et al., 2012).

In our earlier study, a nanoformulation of glycyrrhizin was first reported and tested against two Gram-negative and two Gram-positive bacteria; the results were then interpreted to assess the potential of excipient screening techniques for improving the entrapment efficiency of hydrophilic drugs (Rani et al., 2015). In this study, as part of our continuing efforts to assess the efficacy of nanoformulations of hydrophilic drugs, we focused on the anti-hyperglycemic effect of glycyrrhizin compared to a standard anti-diabetic drug, metformin, in a rat model of type-II diabetes.

2. Materials and methods

2.1. Drugs and chemicals

Glycyrrhizin, streptozotocin (Sigma Chemicals Co., St. Louis, MO, USA), chitosan, gum arabic, polysorbate-60, nicotinamide, and metformin (Hi-Media Laboratories Pvt. Ltd., Mumbai, India) were purchased and used in this study. All other chemicals used in the experiments were of reagent grade. Female albino Wistar rats, weighing 140–180 g, were procured from the Disease-free Small Animal House at Lala Lajpat Rai University of Veterinary and Animal Sciences (Hisar, Haryana, India). The rats were housed under standard environmental conditions with a 12-h light/12-h dark cycle and fed a pellet diet (Ashirwad Industries, Mohali, Punjab, India) with free access to water. Prior approval for the experimental protocol was given by the Institutional Animal Ethics Committee in its 25th meeting held on December 19th; 2013. The rats were cared for per the CPCSEA guidelines from the Indian Ministry of Environment, Forest & Climate Change.

2.2. Preparation of glycyrrhizin- and metformin-loaded NPs

The nanoformulations were successfully prepared *via* the ionotropic gelation method using chitosan and gum arabic as the basic polymers for encapsulation of glycyrrhizin. The polymer concentrations were optimized for particle size and encapsulation efficiency using the two-factor, three-level (3^2) factorial method in the Design Expert program (Version 8.0.7.1). Glycyrrhizin-loaded NPs were synthesized using a dropwise addition of glycyrrhizin solution (1/7th of polymer) into a chitosan solution (1–1.5% w/v) which had been prepared in 2% (v/v) acetic acid. To this solution, gum arabic solution (0.1–0.2% w/v) was added slowly with continuous stirring followed by the addition of polysorbate-60 (1% v/v of the whole solution). For the preparation of NPs, the optimized concentrations of chitosan and gum arabic polymers were determined to be 1.3% w/v and 0.15% w/v respectively (Rani et al., 2015).

The optimized batch conditions for the preparation of glycyrrhizin-loaded NPs were used for the formulation of metformin-loaded NPs. Metformin-loaded NPs were synthesized using a dropwise addition of metformin solution (1/7th of polymers) into a chitosan solution (1.3% w/v) that was initially prepared with 2% (v/v) acetic acid. Next, gum arabic solution (0.15% w/v) was added with constant stirring. Finally, polysorbate-60 (1% v/v of the whole solution) was added slowly to the whole mixture. Fig. 1 shows a schematic of the synthesis of glycyrrhizin and metformin NPs. Blank nanoparticles were prepared in a similar fashion, but without the addition of drug (glycyrrhizin or metformin) to the chitosan solution. The nanosuspension was mixed with 1% *w/v* mannitol as a cryoprotectant and lyophilized using a lyophilizer (Alpha