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Optimization of processing parameters for the development of *Ficus religiosa* L. extract loaded solid lipid nanoparticles using central composite design and evaluation of antidiabetic efficacy

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ABSTRACT

The aim of the present work was to optimize processing parameters for the preparation of *Ficus religiosa* L. extract loaded solid lipid nanoparticles (SLN) using central composite design. Optimization was carried out using four factors such as homogenization speed, homogenization time, sonication time and sonication intensity and three responses studied were particle size, polydispersity index (PDI) and zeta potential to obtain a SLN batch with lesser particle size, optimum PDI and higher zeta potential. Further, optimized batch was characterized for entrapment efficiency, surface morphology, *in-vitro* release and kinetics, fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), powder Xray diffraction (PXRD) and stability over 6 months. From the results, it was observed that increase in homogenization speed and time decreased particle size and PDI with increase in zeta potential value. Increase in sonication time up to 5 min decreased particle size and further increase had no effect on particle size. Sonication intensity had very little effect. DSC and PXRD showed reduced crystallinity of extract in SLN form. Also, the optimized batch had stability over 6 months. Further, SLN significantly reduced diabetes induced higher levels of blood glucose and increased diabetes induced lower level of plasma insulin.

1. Introduction

Ficus religiosa L. is known as peepal tree and it is a sacred tree in India. It belongs to the family Moraceae. Several parts of *Ficus religiosa* L. tree such as barks, roots, leaves, fruits, latex and decoction are reported to possess pharmacological benefits. *Ficus religiosa* L. possesses strong anti-oxidant activity and it is used in the treatment of diabetes, neuronal disorders and inflammatory conditions and used against numerous microbes. *Ficus religiosa* L. have been included in several ayurvedic formulations [1,2]. SLN can be defined as colloidal drug delivery system consisting of solid lipid and stabilized with surfactant and particle size ranging from 10 nm to 1000 nm [3]. In recent years, SLN are being used for entrapping natural drugs either as crude drug or extract or single phytochemical in improving the properties of entrapped drug [4].

For the preparation of SLN, several factors are to be considered which include effect of lipids and surfactants [5], processing parameters such as homogenization and sonication, formulation parameters such as drug to lipid ratio, lipid type and concentration and surfactant type and concentration [6]. Processing parameters have greater influence on particle size, PDI and zeta potential which eventually affect the stability of particles during storage. In case of processing parameters effect, two techniques are widely used to disperse the particles which include high shear homogenization and ultrasonication [7,8]. Both have greater effect on size and charge of particles. Hence, it is necessary to optimize these two parameters to obtain SLN with lesser particle size, optimum PDI and higher zeta potential. For optimization, effect of different variables on nanoparticles should be considered and optimization is done by changing single variable and keeping other variables as constants. This is a time consuming process and also it does not allow studying the combined effect of variables. A factorial design approach or response surface methodology (RSM) is applied to minimize number of experiments and to study the combined effect of variables/factors together which is time effective. RSM is a

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Abbreviations

CCD	Central composite design
RSM	Response surface methodology
VIF	Variance inflation factors

collection of statistical and mathematical techniques useful for developing, improving and optimizing processes. It is widely used in the design, development and formulation of new products/formulations, and improvement of existing product design [9]. Optimization of SLN loaded with drug(s) is very important as each drug vary with their physiochemical properties which might have influence on size and charge of SLN.

In the present study, optimization of *Ficus religiosa* L. extract loaded SLN was done by using central composite design (CCD). Factors studied include homogenization speed, homogenization time, sonication time and sonication intensity and the responses studied include mean particle size, PDI and zeta potential. Additionally, the optimized batch was characterized for entrapment efficiency, surface morphology, *in-vitro* release and kinetics, solid state characterization by fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), powder Xray diffraction (PXRD) and stability studies. And the optimized batch was evaluated for antidiabetic efficiency.

2. Materials and methods

2.1. Materials

Standard, lupeol (95% purity) was procured from Sigma Aldrich, India. Glyceryl monostearate, poloxamer 188 and sodium deoxycholate were kind gifts from Hospira Pvt. Ltd, Chennai, India. Dialysis membrane (cutoff MW 12,000–14,000 daltons) was procured from HiMedia, India. Water used in all experiments was purified by Milli-Q-plus system (Millipore, India). All other chemicals and solvents were of analytical grade.

2.2. Isolation of ethanolic extract of Ficus religiosa L

The stem barks of *Ficus religiosa* L. were collected in the month of November from the locality of Banaras Hindu University (Varanasi, India) and authenticated by Prof. R. S. Upadhyay, Department of Botany, Banaras Hindu University, Varanasi, India. Stem barks of *Ficus religiosa* L. were dried under sun for 15 days and powdered finely. 50 g of this powder was taken into the porous container of soxhlet apparatus. 500 ml of ethanol was used for extraction; 250 ml of ethanol was taken in distilling pot and remaining ethanol was poured to porous container. Temperature of 40 °C was maintained and soxhleted for 48 h. Then the solvent was recovered and extract was dried. Traces of organic solvent were completely removed at 70 °C using rotary evaporator (IKA RV 10) by nitrogen gas purging. Dried ethanolic extract was used for the study. TLC was performed by using Silicagel G as adsorbent and n-butanol: acetic acid: water (4:0.5:5) as mobile phase. Plate was prepared by pouring silica gel slurry on glass plate and activated by heating at 110 °C for 30 min. The spots were detected using vanillin reagent in sulphuric acid and R_f values were calculated. Preparative TLC using 60F254 precoated TLC plates (Merck) of 20 × 20 cm dimension was carried out by partitioning 25 g of ethanolic extract with 50 ml of petroleum ether. This extract was then concentrated and used for isolating marker compound.

2.3. Standardization of ethanolic extract of Ficus religiosa L

For qualitative analysis, LCMS was carried out by using TOF/Q-TOF Mass spectrometer (Agilent technologies), equipped with a electrospray ionisation source in both positive and negative modes. Minimum range and maximum range of 50 and 1500, respectively were used. Other parameters such as scan rate of 1, gas temperature of 300 °C, gas flow of 10.0 l/minute and nebulizer pressure of 32 psi were used. FTIR analysis of marker compound was done by using FTIR (FTIR-8400S, Shimadzu) by conventional KBr disc/pellet method. The sample was prepared by grinding with anhydrous KBr powder and compressed into pellets. An FTIR spectrum was measured over the range of 4000 - 400 cm^{-1} with resolution of 4 cm^{-1} for 50 scans. Structural elucidation of marker compound was done by using ^{1}H NMR and ^{13}C NMR analyses. ^{1}H NMR was carried out at 500 mega hertz using tetramethylsilane as internal standard.

2.4. Experimental factorial design

In the present work, optimization of SLN was done by using CCD and the data were analyzed using Design Expert® software (Trial version 9.0.3.1, Stat-Ease Inc., Minneapolis, MN, USA). The selected factors were homogenization speed (A), homogenization time (B), sonication time (C) and sonication intensity (D). Different ranges studied include homogenization speed of 5,000, 10,000 and 15,000 rpm, homogenization time of 15, 30 and 45 min, sonication time of 2.5, 5 and 7.5 min and sonication intensity of 40, 50 and 60% amplitude. Optimization was performed to determine the optimal levels for lesser mean particle size (Y1), optimum PDI (Y2) and higher zeta potential (Y3). According to CCD, each factor was set to 5 levels: plus and minus alpha (axial points), plus and minus 1 (factorial points) and the centre point with α-value of 2 before generating the experimental design. The software had generated a total of 30 runs (Table 1). The second-order polynomial equations were used to express the mean particle size, PDI and zeta potential of the SLN.

2.5. Preparation of solid lipid nanoparticles

SLN were prepared by the combined method of hot homogenization and ultrasonication. Lipid was melted and extract was dispersed in the melted lipid phase. Aqueous phase containing surfactant was poured into the lipid phase which was maintained at the same temperature of lipid phase. 2% lipid concentration, 0.5% poloxamer 188, extract to lipid ratio of 1:5 were selected. Homogenization was performed at different speeds and different times using high speed homogenizer (T25, Ultraturrex, IKA). SLN suspension was then allowed to cool to room temperature. Then, formed suspension was sonicated at different times and different intensities by using ultra-sonicator (200H, Hielscher).

2.6. Particle size, PDI and zeta potential

Particle size, PDI and zeta potential of SLN formulations were measured by using dynamic light scattering (Delsa™Nano C, Beckman coulter) technique. Samples were measured at a fixed angle of 165° at 25 °C for particle size and PDI analyses. Zeta potential of SLN was measured at 25 °C. Average particle size, PDI and zeta potential were measured in triplicate.

2.7. Entrapment efficiency

1 ml of SLN dispersion was taken in eppendroff tubes and centrifuged in a high speed cooling centrifuge (C-24, Remi) at