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Formulation and development of Silybin loaded solid lipid nanoparticle enriched gel for irritant contact dermatitis

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Glyceryl monosterate (Pubchem CID: 24699)
Compritol (Pubchem CID: 62726)
Cetyl palmitate (Pubchem CID: 10889)
Steric acid (Pubchem CID: 5281)
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Dinitrochlorobenzene [DNCB] (Pubchem CID: 6)
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ABSTRACT

The purpose of the present research was to develop Silybin loaded solid lipid nanoparticle gel (SIL-SLN gel) for irritant contact dermatitis (ICD). ICD is associated with reduced skin water content, emerging in dry skin condition and relapsing eczema. SIL is a naturally occurring flavones, which shows antioxidant activity and helps in the treatment of ICD. In this study, the SLN was prepared by the ultrasonic probe sonication method and further evaluated for particle size and entrapment efficiency. Results of optimized batch showed mean particle size 139 ± 0.35 nm and entrapment efficiency 90.97 ± 0.91%. Optimized batch was freeze dried and characterized by field emission scanning electron microscopy (FE-SEM), it shows particles are in nano range, with spherical morphology and smooth surface. Finally, the SLN was incorporated into gel for convenient topical application. The SIL-SLN-gel were evaluated for *in-vitro* skin occlusivity, skin irritation and *ex-vivo* diffusion and deposition study and further compared with SIL-plain gel. Efficacy of gel on dinitrochlorobenzene (DNCB) induced ICD mice were evaluated by skin water content, ear swelling and histopathology. Ex-vivo study of SIL-SLN gel exhibited prolonged drug release, whereas the skin irritation study shows no irritancy. In DNCB induced ICD mice SIL-SLN gel showed higher efficacy than SIL-plain gel.

1. Introduction

Skin is the largest organ of the human body often exposed to the external environment; as a result, there is uncertainty of occurrences of various skin diseases like dermatitis, psoriasis, warts, tineapedis and eczema, etc. Irritant contact dermatitis (ICD) is a potential devitalizing chronic skin disorder that has a significant impact on human population worldwide. Occurrence of ICD has risen in recent years imposing high economic burden on many developing countries [1–4]. The idiosyncratic symptoms of ICD include: mild to severe erythema, oedema, itching, epidermal thickening and scaling [5]. In most cases, the ICD is associated with reduced skin water content, emerging in dry skin condition and relapsing eczema [6]. Due to the dryness, skin become permeable and susceptible to adverse environmental factors that leads to entry of irritant and allergens into the skin, which occasionally act as triggers and can provoke allergy with another episode of inflammation [7,8]. There are handful treatments available for ICD such as pharmaceutical formulation containing antihistamines and steroids; but long term use of these can lead to several side effects

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[9]. So, to overcome these, it is necessary to replace the synthetic drugs with herbal extract as these have several advantages like low cost, no side effects, easy availability, low toxicity etc. Enormous work has been already done on plant extract in the treatment of skin diseases like dermatitis, psoriasis and wound healing etc., but very few of them are successful due to challenges like assurances of safety, quality and efficacy of medicinal plants and herbal extracts during commercialization process. Similarly, multi-component effect of herbal extract deteriorates the herbal activities as each component in extract has different stability conditions [10,11]. So, to avoid this while formulating, it is essential to use single isolated herbal moiety. Silybin is a naturally occurring flavone and it is active constituent of silymarin, a flavonolignan from 'milk thistle' (*silybum marianum*), it is widely recognised for its hepatoprotective activity [12]. Numerous studies suggest that Silybin is powerful antioxidant and it has anti-inflammatory properties which can be used in treatment of dermatitis [13]. As an antioxidant, Silybin can reduce the oxidative damage caused by pathogens in the deep layers of the skin. The anti-inflammatory effect of silybin can also reduce local inflammation in the skin by using topical delivery system, as it is suitable for treatment of skin diseases. Topical treatment is most promising approach for the treatment of skin diseases due to lower risk of systemic side effects and higher retention of drug can be achieved at the site of disease. However, drugs present in the conventional formulations like creams, gels, lotions, etc. cannot deliver the actives in required amount at the target site. So, the selection of suitable carrier is the most significant aspect, as the carrier determines the performance of the formulation. Thus, the formulator should select a suitable carrier, which should increase the drug deposition in the skin strata, as well as it should protect drugs from photo degradation. For improving delivery of drug to the skin number of innovative micro particulate carrier systems viz: liposomes, micro emulsions, nano emulsions, solid lipid nanoparticles, nanostructured lipid carrier, etc. have been reported [14]. SLNs are a colloidal carrier system for controlled drug delivery and it is widely used in the topical formulations [15]. Due to their nanosize particles are able to increase the penetration, occlusion and accumulation of drug in the dermis. Solid lipid matrix controlled the release of drug from these carriers. To get a topical dosage form having the desired semisolid consistency, the SLN dispersion can be incorporated into gels or creams. Gel normally preferred because of their controlled release character and good compatibility with the tissues.

Thus, the objective of present work is to formulate of Silybin loaded solid lipid nanoparticles enriched gel and to investigates its use in treatment of ICD by using *in-vitro* and *in-vivo* studies.

2. Material and method

2.1. Materials

SIL was procured from Prolab Pvt. Ltd. (New Delhi), Tween 20, Tween80, Span 20, Triton x-100 and Span 80 were gifted by LobaChemie Pvt. Ltd. (Mumbai), Cetyl palmitate, steric acid, compritol, Glyceryl monosterate and Precirol ATO5 was gifted by Gattefosse (Mumbai), Carbopol 940 was gifted by Oxford Laboratory (Mumbai) and dinitrochlorobenzene [DNCB] was purchased from Panoli Intermediates Pvt. Ltd. (Mumbai) India. All the reagents and excipient were used as received.

2.2. Animals

All experimental procedures and protocols used in this study were reviewed and approved (SCOP/IAEC/Approval/2014-15/199) by the 'Institutional animal ethics committee' (IAEC) of Sinhgad college of pharmacy, Pune, constituted under 'Committee for the purpose of control and supervision of experiments on animals' (CPCSEA). Animal care and handling throughout the experimental procedure were performed in accordance to the CPCSEA guidelines. White New Zealand rabbits weighing 2.5–3 kg and healthy Balb/c mice of either sex 25–30 g were obtained from the animal house of (National Institute of Biosciences, Pune, India) and were used for *in-vivo* skin irritancy and anti-dermatitis activity respectively.

2.3. Screening of components (solubility studies)

This study was done for determining the solubility of the drug in the components to be used in the formulation, namely, solid lipids and surfactants. An array of solid lipids and surfactants are subjected to this study to select the most compatible out of each category. Solubility of drug in various lipids was analyzed in order to select the lipid having a maximum potential to solubilize the drug [16]. Various lipids such as cetyl palmitate, Compritol 888 ATO, glyceryl monostearate, Precirol ATO5, Emulsire and stearic acid were used to study the solubility of the drug. Briefly, SIL (10 mg) was added to a glass vial. Lipid was added to the vial in gradually increasing amount. The above mixture was heated to a temperature above 5–10 °C of the lipid's melting point. A transparent solution of the drug and melted lipid indicates solubilization of the drug in the lipid. This serves as an end point. The amount of each lipid added was calculated. In contrast to this, for surfactant an excess amount of SIL was added to a known amount of the surfactant and mixed for 2 min, followed by sonication for 10 min to dissolve the drug. Mechanical shaker was further used for 8–12 h to dissolve the drug. The mixture was then centrifuged at 15,000 rpm for 15 min. The aliquots of supernatant saturated surfactant systems were diluted appropriately and analyzed using HPLC. Tween 20, Tween 80, Triton X100, Labrasol, Span 20 and Span 80 were used for surfactant solubility.

2.4. HPLC method development for silybin

The quantitative determination of SIL was performed by high performance liquid chromatography (HPLC) (Shimadzu, LC 2010) at 288 nm. Briefly, samples were chromatographed on a 4.6 mm × 250 mm reverse phase stainless steel column packed with 5 μm particles (Hypersil C18) and eluted with a mobile phase consisting of methanol: water in the ratio of 50:50 v/v at a flow rate of 1 ml/min.

2.5. Development of SIL loaded SLN's by ultrasonic probe sonication method

The Precirol ATO5 and Tween 20 were selected as solid lipid and hydrophilic surfactant respectively for preparation of SLNs based on their high solubilizing capacity of silybin. Amount of lipophilic surfactant i.e. Span-80 (1 ml) and SIL (50 mg) was kept constant in all nine batches for production of 50 ml of SLN batch. Tween 20 as a long chain surfactant provides aqueous phase stability to the formed emulsions and Span 80 as a lipophilic surfactant improves the entrapment efficiency of drug, so it was used in the formulation of SLNs. The SLNs were prepared by homogenization followed by ultrasonic probe sonication method [17]. The SIL and lipophilic surfactant (Span 80) were added in melted solid lipid (Precirol ATO5). The aqueous surfactant (Tween 20) solution was prepared separately in 50 ml water. Both mixtures were heated in the controlled temperature water bath (at 55–60 °C higher than melting point of lipid to avoid solidification of lipid). The aqueous surfactant solution was added in the lipidic mixture at the same temperature and stirred continuously using a high speed