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Delivery of adapalene using a novel topical gel based on tea tree oil nano-emulsion: Permeation, antibacterial and safety assessments

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ABSTRACT

The aim of present study was to design and optimize 0.1% adapalene loaded nano-emulsion to improve the drug efficacy and increase its user compliance. Effect of type and concentration of surfactants was studied on size of 0.1% adapalene loaded nano-emulsion. Optimized formulation was then evaluated for particle size, polydispersity index, morphology, viscosity, and pH. Subsequently, 1% carbopol® 934 was incorporated to the optimized formulation for preparation of its gel form. The efficacy and safety of 0.1% adapalene loaded nano-emulsion gel was assessed compared to marketed gel containing 0.1% adapalene. In-vitro studies showed that adapalene permeation through the skin was negligible in both adapalene loaded nano-emulsion gel and adapalene marketed gel. Furthermore, drug distribution studies in skin indicated higher retention of adapalene in the dermis in adapalene loaded nano-emulsion gel compared with adapalene marketed gel. Antibacterial activity against Propionibacterium acnes showed that adapalene loaded nano-emulsion is effective in reducing minimum inhibitory concentration of the formulation in comparison with tea tree oil nano-emulsion, and pure tea tree oil.

In vivo skin irritation studies showed absence of irritancy for adapalene loaded nano-emulsion gel. Also, blood and liver absorption of the drug, histological analysis of liver and liver enzyme activity of rats after 90 days' treatment were investigated. No drug was detected in blood/liver which in addition to an absence of any adverse effect on liver and enzymes showed the potential of adapalene loaded nano-emulsion gel as a novel carrier for topical delivery of adapalene.

1. Introduction

One of the most common dermatological diseases is acne vulgaris which can develop at any age (Nguyen et al., 2016). The disease is characterized by non-inflammatory (open and closed comedones) or inflammatory (papules and pustules) lesions (Shalita, 2004). The main cause of acne vulgaris is an increase in sebum production, which provides a substrate for growth of bacterial flora in the normal skin such as Propionibacterium acnes (*P. acnes*). In fact, colonization of *P. acnes* in pilosebaceous units as well as hyperkeratinization of follicles leading to a blockage of the pilosebaceous channels results in inflammatory and non-inflammatory lesions (Canavan et al., 2016; Williams et al., 2012).

Topical therapy is the first choice to treat mild and moderate acne vulgaris, and in severe cases, it can provide complementary support to other conventionally administered forms of acne vulgaris treatment (Garg, 2016). Topical retinoids including adapalene, tretinoin, isotretinoin, and tazarotene are among popular treatments that act on abnormal keratinization of follicles (Canavan et al., 2016). Adapalene, (6-[3-(1-adamantyl)4-methoxyphenyl]-2-naphthoic acid), is a naphthoic acid derivative that is widely used for topical treatment of acne vulgaris. It acts on comedones by regulating cellular differentiation (Czernielewski et al., 2001; Irby et al., 2008). While adapalene has been reported to be a safe treatment, clinical studies suggest that it significantly improve inflammatory and non-inflammatory lesions (Alirezai et al., 1998; Cunliffe et al., 1998; Diane et al., 2005; Loesche, 1998; Nyirady et al., 2001; Thiboutot et al., 2008). In recent years, several attempts have been carried out on preparation of adapalene-based nano-carriers for topical treatment of acne vulgaris (Guo et al., 2014; Harde et al., 2015; Jain et al., 2014; Prasad et al., 2012; Ramezanli et al., 2017).

In addition to pharmaceutical treatments, essential oils have been used for acne vulgaris treatment for many years. In fact, Australian tea tree oil obtained from steam distillation of *Melaleuca alternifolia* leaves is a well-known substance for treatment of acne vulgaris with potent antibacterial effect (Bassett et al., 1990; Lins et al., 2016). Recent studies showed that nano- and micro-emulsions can be promising techniques to deliver such therapeutics to treat acne vulgaris (Najafi-Taher and Amani, 2017). Tea tree oil nano-emulsions have been previously reported as an effective treatment for psoriasis (Sonia and Anupama, 2011) as well as a local therapy for fungal and bacterial pneumonia (Li

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et al., 2016). Our recent study suggests that tea tree oil nano-emulsion loaded with Ag nanoparticles have synergistic antibacterial effect against *Escherichia coli* (Najafi-taher et al., 2017). In this work, for the first time, adapalene loaded nano-emulsion of tea tree oil was developed and characterized. The optimized formulation was characterized for various parameters like size, morphology, viscosity, stability and skin permeation. Investigations of in-vitro antibacterial activity and in-vivo safety of the preparation were also performed.

2. Materials and methods

2.1. Materials

Adapalene was gifted by Tolid Daru (Iran). Formaldehyde and normal saline were purchased from Ave Sina, (Iran); Adapalene marketed gel (0.1%) was purchased from Aburaihan pharmaceutical Co., Tehran, Iran. All other reagents and materials were purchased from Sigma-Aldrich (USA).

2.2. Preparation of adapalene loaded tea tree oil nano-emulsion

In this study, spontaneous emulsification was employed to prepare 0.1% adapalene loaded tea tree oil nano-emulsion. Initially, an organic phase was prepared, consisting of tea tree oil (6%), adapalene (0.1%), and dimethyl sulfoxide (DMSO) (10%). It was then slowly added into an aqueous phase containing ethanol and different surfactants being stirred at fixed speed using a magnetic stirrer. Different non-ionic surfactants (Tween 20, 60 and 80 as well as Span 80) in concentration of 15% were examined to investigate their influence on nano-emulsion preparation. Subsequently, different concentrations of the surfactant were investigated (5–30%) to reach optimum concentration of the surfactant for preparation of the nano-emulsion. On the basis of the concentration studies, Smix ratio (surfactant/co-surfactant) was chosen to construct pseudo-ternary phase diagram to find nano-emulsion region. The organic phase was slowly added to the aqueous phase. Transparent samples were taken as nano-emulsion samples. Those which remained clear after freeze-thaw cycle (see Section 2.3.5), were considered as stable nano-emulsion samples.

2.3. Characterization of prepared nano-emulsion

2.3.1. Dynamic light scattering (DLS) and zeta potential analysis

Particles diameter and size distribution of adapalene loaded tea tree oil nano-emulsion were examined by dynamic light scattering (DLS). Nano-emulsions were diluted (4:1) with water prior to the experiment. Zeta potential of adapalene loaded tea tree oil nano-emulsion was determined using Zetasizer (Nano Z-S; Malvern Instruments, UK). All the measurements were performed in triplicates.

2.3.2. Transmission electron microscopy (TEM)

Size, size distribution and morphology of the prepared adapalene loaded tea tree oil nano-emulsion were examined by TEM (100 kW, LEO, Germany). Samples for TEM were prepared with solvent evaporation at ambient temperature.

2.3.3. Viscosity

The viscosity of the samples was evaluated without further dilution by Brookfield digital viscometer (China). All the experiments were performed in triplicates.

2.3.4. pH

pH of adapalene loaded tea tree oil nano-emulsion was tested using a calibrated potentiometer (Inolab pH 720, WTW, Germany), at ambient temperature. The measurements were conducted 3 times.

2.3.5. Stability assessment

Three freeze–thaw cycles were performed for the formulations between −21 and +25 °C temperature, with storage time at each temperature for 48 h. Also, stability assessment was conducted by keeping the formulations at room temperature over a period of 2 months (Dasgupta et al., 2013).

2.4. In-vitro study

2.4.1. Antimicrobial activity

Antibacterial activity of pure tea tree oil, adapalene loaded tea tree oil nano-emulsion and tea tree oil nano-emulsion against *P. acnes* (ATCC 6919) were examined using various concentrations of the oil (0.2–10 mg/ml). The bacteria were cultivated for 48 h in anaerobical conditions in brain heart infusion (BHI) broth with 1% glucose. 100 µl of each formulation was added to 100 µl of bacterial suspension (1×10^8 cfu/ml) in each well. Absorption was measured at 600 nm.

2.5. Ex-vivo study

2.5.1. Drug permeation

Permeation studies were carried out using shaved skins of rat and dialysis membrane. All experiments were performed based on guidelines for care and use of laboratory animals and approved by the Ethics Committee of Tehran University of Medical Sciences. Fat tissues below the skin were chopped, and skin was rinsed with saline. Skin and dialysis membrane were fixed on vertical Franz diffusion cell. Receptor compartment was filled with PBS (pH 5.6):Acetonitrile:THF (3:10:2) and stirred at 300 rpm. Cells were kept at 32 ± 0.5 °C. 1% carbopol® 934 was used to prepare gel from adapalene loaded tea tree oil nano-emulsion and tea tree oil nano-emulsion. Adapalene loaded tea tree oil nano-emulsion gel, tea tree oil nano-emulsion gel, and adapalene marketed gel were applied in the donor compartment. 0.1% adapalene solution in Acetonitrile:THF (5:1) was used as a reference to compare permeation ability of the formulations. Tea tree oil nano-emulsion gel was used as blank to remove possible effect of components of nano-emulsion in the detection of adapalene. 0.5 ml sample from the receptor compartment was collected at 0.5, 1, 2, 4, 6 and 8 h, and the same volume of the solvent was added to the receptor compartment (Martins et al., 2011). All samples were filtered through an aqueous 0.44 µm pore size cellulose membrane filter. The concentration of adapalene was assessed by high-performance liquid chromatography (HPLC) method as reported earlier with slight modifications. Briefly, HPLC analysis was performed using C18 column (250 mm × 4.6 mm, particle size 5 µm) and acetonitrile–water (90:10, pH 16 was adjusted to 3 with Orto-phosphoric acid) as the mobile phase (flow rate: 1.3 ml/min) at 40 °C for 20 min. Injected volume was 25 µl for all solutions, and detection wavelength was set at 235 nm (Liu-Jiang and Xiao-Fen, 2007; Martins et al., 2011).

2.5.2. Skin distribution

After performing in-vitro permeation studies, skin was washed three times with deionized water and let dried. Epidermal and dermal layers were separated with tweezers. Then, chopped into pieces and put in acetonitrile:THF (5:1) to extract adapalene (Liu-Jiang and Xiao-Fen, 2007). Solutions were filtered through a membrane (0.44 µm) and analyzed for adapalene.

2.6. In-vivo studies

2.6.1. Skin irritation study

Skin irritation study was conducted in 12 healthy New Zealand white rabbits (1500 ± 500 g b.w.). Adapalene loaded tea tree oil nano-emulsion gel, tea tree oil nano-emulsion gel, and adapalene marketed gel were applied over the shaved area. 1% carbopol® 934 gel was used as negative group. The treated sites were covered with gauze. The