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Transdermal potential and anti-arthritic efficacy of ursolic acid from niosomal gel systems

Mahvish Jamal [a], Syed Sarim Imam [b], Mohd. Aqil [a], Mohd. Amir [a], Shaukat R. Mir [a], Mohd. Mujeeb [a,*]

[a] Faculty of Pharmacy, Jamia Hamdard University, New Delhi, India
[b] Glocal School of Pharmacy, Glocal University, Saharanpur, Uttar Pradesh, India

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ABSTRACT

The aim of the present study was to optimize niosomes by experimental design for enhanced transdermal delivery of ursolic acid for the effective treatment of arthritis. The experimental design (3 factor 3 levels, Box–Behnken design) was used to study individual and combined effects of different formulation variables. The variables cholesterol (X_1), span 60 (X_2) and phospholipid (X_3) were taken as independent factors and their effect was observed on size (Y_1) entrapment efficiency (Y_2), and transflux (Y_3). The formulation composition with span 60 (85 mg), cholesterol (12.3 mg), and phospholipid (65 mg) was found to fulfil requisites of optimized ursolic acid niosome formulation (URNF). URNF had shown vesicle size of 665.45 nm, entrapment efficiency of 92.74% with transflux of 17.25 μg/cm^2/h. The in vivo bioactivity showed that the prepared URNF-gel was able to provide good anti-arthritic activity due to enhanced permeation of UA through the skin and results were found to be comparable to standard gel (Omni gel). The radiographical image confirmed that, the developed URNF-gel was found to be effective to treat arthritis. Thus niosomal gel of ursolic acid would be a promising alternative to conventional therapy for safe and efficient treatment of arthritis and musculoskeletal disorders.

1. Introduction

Ursolic acid (UA, Fig. 1), is a constituent of *Lantana camara* (Verbenaceae) and possesses high medicinal value. It is used in several herbal medicines marketed in Asia and worldwide for inflammatory conditions [1–3]. Chemically, it is a pentacyclic triterpenoids having chemical name (3ß-hydroxy-urs-12-en-28oic acid) and used for various pharmacological activities such as anti-inflammatory activity [4,5], trypanocidal, antiviral, antioxidant, and anti tumour activities [6]. It possesses strong anti-inflammatory activity by inhibiting the activity of COX-2, also inhibiting the TNF-induced activation of NF-κB in Jurkat cells as well as NF-κB transcription in human T lymphocytes [4,7]. The therapeutic efficacy of UA is limited due to its poor oral bioavailability. The poor oral bioavailability of UA has been attributed to its poor aqueous solubility and extensive first pass metabolism [8]. Therefore, for effective treatment of arthritis there is a need to develop a formulation to enhance the bioavailability and therapeutic efficacy. Regrettably, oral formulations have a number of limitations such as extensive first pass metabolism and gastrointestinal irritation. For these reasons, advanced localized and transdermal delivery has gained a lot of importance these days [9,10]. Diverse approaches intended for enhanced transdermal delivery of herbal drugs like curcumin nano-emulsion systems [11], curcumin ethosomes [12], luteolin niosomal gel system [13], diclofenac and curcumin transfersomes [14,15] and ursolic acid topical formulation [16], to list a few. An efficient and secure topical formulation has noteworthy applications in treating varied disorders and clinical conditions owing to the facts such as bypass of gastrointestinal disturbances, diminished renal toxicity, reduced dose frequency and increase patient compliance [17,18,19]. The conventional transdermal formulation typically provides relatively high concentrations of the drug, but for a short duration of time; leading to a cycle of short term over medication followed by long-term under medication. So, formulation of novel transdermal system is necessitated, which will increase the presence of active agents either on the skin surface or within epidermis concurrently reducing hasty penetration [17,20]. Proniosomes are liquid crystalline-compact niosomal hybrid which could be converted into niosomes upon hydration with water offering a versatile transdermal lipid vesicle delivery. The phospholipids and non-ionic surfactants present in proniosomes can act as penetration enhancers, since it was found that some phospholipids are able to fluidize the stratum corneum lipid bilayers [21]. These systems have been reported to decrease side effects and to provide a considerable drug release [22], thus, taking the advantages of niosomal transdermal drug delivery system, disadvantages of conventional topical dosage forms, and non availability of niosomal gel of ursolic acid. The present research work was aimed to formulate and evaluate ursolic acid niosomal gel for its promising effects over an extended period of time. This study describes a more

* Corresponding author at: Faculty of Pharmacy, Jamia Hamdard University, New Delhi, 110062, India.
E-mail addresses: aqilmalik@yahoo.com (M. Aqil), drmmujeeb12@gmail.com (M. Mujeeb).

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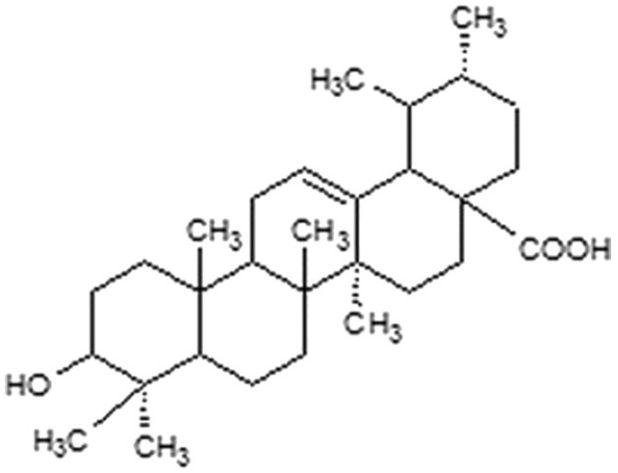

Fig. 1. Chemical structure of ursolic acid.

effective and efficient strategy to deliver ursolic acid via the transdermal route at the affected joint area for prolonged period to treat effectively the inflammation and other associated symptoms or progression of cartilage degradation associated with arthritis.

2. Material and methods

2.1. Materials

UA was purchased from Yukka Pharmaceuticals, India. Span 60, Cholesterol, Carbopol-943 were provided by S.D. fine chemicals Limited Mumbai, India. Phospholipon®G90 was provided as gratis sample by Phospholipid GmbH (Nattermannallee, Germany). Complete Freund's adjuvant (CFA) was purchased from MP Biomedicals Pvt. Ltd., Mumbai, India. Water for high- performance liquid chromatography was provided by Thomas Baker Chemicals Ltd. (Mumbai, India). The standard gel (Omnigel, 1%; Cipla Ltd., Baddi, Himachal Pradesh, India) was used for biological study. All other chemicals used were of analytical grade.

2.2. Formulation of niosomes

UA loaded niosomal formulations were prepared by film hydration technique using non-ionic surfactant [21]. The formulations were prepared by taking calculated amount of cholesterol, phospholipids and span 60 in a solvent mixture (chloroform:methanol, 1:1) in a dry round-bottom flask. Calculated amount of ursolic acid was dissolved in the above mixture and organic phase was evaporated using a rotary vacuum evaporator (HS-2005 V-N; Hahnshin Scientific Co., South Korea) to form a thin film on the wall of the flask. The flasks were kept in a desiccator under vacuum for 24 h to ensure complete removal of trace solvents. The proniosomes were converted to niosomes by hydrating the film using 10 mL phosphate buffer at 55 °C and sonicated (Titanium probe, Ultrasonicator, Model-UP100H; Hielscher Ultrasonics GmbH, Berlin, Germany) for 1 h. The niosomal suspension was left to mature overnight at 4 °C and stored at refrigerator temperature for further studies. The experimental design was used to optimize the developed formulation and the fifteen formulations of different composition were shown in Table 1.

2.3. Experimental design

The design matrix was used to explore the quadratic response surfaces and for constructing a second-order polynomial models using Design Expert software (Version 8.1.6; Stat-Ease Inc., Minneapolis, MN, United States). The experimental runs for which the nonlinear computer generated quadratic model are defined as:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}{X_1}^2 + b_{22}{X_2}^2 + b_{33}{X_3}^2$$

Where Y is the measured response associated with each factor level combination; b_0 is constant; X_1, to X_3 are coded levels of independent variables. The terms X_1X_2 and X_i^2 (i = 1, 2, or 3) represent the interaction and quadratic terms, respectively [23]. The different concentration range of cholesterol (X_1), span 60 (X_2), phospholipid (X_3), and their respective observed and predicted responses are shown in Table 1.

2.4. Quality evaluation

2.4.1. Vesicle size and size distribution

The size distribution was measured by Dynamic Light Scattering (DLS) technique using Malvern Instruments (HAS 3000, United Kingdom). 1-mL sample was diluted with double distilled water and measurements were taken in triplicate. The polydispersity index (PDI) was determined as a measure of homogeneity. Small values of PDI

Table 1
Experimental runs obtained from Box–Behnken design and their actual and predicted response in Box–Behnken design for ursolic acid niosomal formulation.

	Independent variable			Dependent variable					
	X_1 (mg)	X_2 (mg)	X_3 (mg)	Y_1 (nm)		Y_2 (%)		Y_3 (μg/cm²/h)	
Run				Actual	Predicted	Actual	Predicted	Actual	Predicted
1	15	65	60	558.98 ± 1.45	556.14	69.87 ± 3.45	68.38	8.44 ± 1.65	9.65
2	10	80	60	490.89 ± 1.12	491.45	86.87 ± 2.76	89.76	14.29 ± 1.87	13.76
3	10	80	60	493.78 ± 1.22	494.54	86.45 ± 1.66	84.87	14.35 ± 1.58	14.19
4	15	95	60	665.45 ± 1.25	666.61	86.98 ± 4.45	85.87	15.34 ± 1.91	15.12
5	5	80	80	436.78 ± 0.75	437.59	84.34 ± 3.47	86.38	10.87 ± 1.67	10.14
6	5	80	40	446.32 ± 0.87	445.78	71.76 ± 3.66	70.52	9.63 ± 1.23	10.65
7	15	80	40	667.91 ± 0.38	668.59	79.45 ± 2.12	77.54	12.68 ± 1.13	11.85
8	10	95	40	625.56 ± 0.26	623.67	82.65 ± 4.87	80.63	12.83 ± 1.18	13.76
9	15	80	80	563.38 ± 0.87	562.87	78.32 ± 3.43	79.78	11.55 ± 0.84	10.61
10	5	95	60	448.88 ± 1.11	445.27	89.56 ± 2.21	90.54	11.21 ± 0.67	10.67
11	5	65	60	431.56 ± 1.09	431.96	64.34 ± 4.27	65.84	08.78 ± 0.88	8.13
12	10	65	40	505.98 ± 1.19	505.13	57.33 ± 3.33	55.78	07.72 ± 0.96	7.29
13	10	95	80	508.98 ± 1.21	510.21	84.21 ± 2.67	82.76	12.48 ± 0.93	13.78
14	10	80	60	492.43 ± 0.78	495.67	87.67 ± 3.76	88.51	14.31 ± 0.72	13.67
15	10	65	80	405.55 ± 0.56	404.86	66.32 ± 3.32	66.11	08.23 ± 1.12	9.27
16	Liposomes			476.34 ± 1.87		73.65 ± 2.65		3.56 ± 0.67	

X_1 = Cholesterol, X_2 = Span60, X_3 = phospholipid.
Y_1 = size (nm), Y_2 = Entrapment efficiency (%), Y_3 = flux (μg/cm²/h).