Colloids and Surfaces B: Biointerfaces 116 (2014) 183–192

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Development and characterization of two nano-structured systems for topical application of flavanones isolated from *Eysenhardtia platycarpa*

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ARTICLE INFO

Article history:
Received 26 July 2013
Received in revised form
14 November 2013
Accepted 5 December 2013
Available online 6 January 2014

Keywords:
Flavanones
Eysenhardtia platycarpa
Polymeric nanoparticles
Nanoemulsion
Skin permeation
Anti-inflammatory activity

ABSTRACT

Many of the inflammatory diseases are becoming common in ageing society throughout the world. The clinically used anti-inflammatory drugs suffer from the disadvantage of side effects. Alternative to these drugs are natural products, since ancient times traditional medicines are being used for the treatment of inflammation. In the present study, four flavanones isolated from *Eysenhardtia platycarpa* leaves with a potent pharmacological activity were formulated in effective drug delivery systems: nanoemulsion and polymeric nanoparticles for topical use as novel anti-inflammatory topical formulations. Nanoemulsion system exhibited droplet sizes less than 70 nm and polymeric nanoparticles with a size of 156–202 nm possessed zeta potential values less than −25 mV that provided good stability and obtained high entrapment efficiency (78–90%). *In vitro* release and *ex vivo* permeation studies were performed on Franz-type diffusion cells and quantified by high performance liquid chromatography (HPLC), all formulations showed steady state release profiles over time and steady increase of flavanones in the skin permeation test. The anti-inflammatory activity, tested by TPA (12-O-tetradecanoylphorbol-13-acetate), induced oedema in mice ear suggesting that prenylated flavanones improve significantly their anti-inflammatory activity when are vehiculized in nanosized systems. Our results suggested that 5-hydroxy-7-methoxy-6-prenyl flavanone loaded nanoemulsion and polymeric nanoparticle could be proposed as potential topical anti-inflammatory formulations with the best properties for the treatment of inflammatory disorders.

1. Introduction

Many of the chronic inflammatory diseases, such as rheumatoid arthritis or systemic lupus erythematosus are becoming common in ageing society throughout the world. Among drugs used to treat different rheumatic diseases anti-inflammatory agents play an important role in improving quality of life of these patients. However, the clinical use of anti-inflammatory drugs for prolonged periods is usually associated with an increased risk of side effects. An alternative to these drugs are natural products, since ancient times, traditional medicines have increasingly been used for the treatment of inflammation.

Eysenhardtia platycarpa, a member of the Leguminosae family known as "taray", "palo dulce" (sweet wood) and "palo azul" (blue wood) is a small tree that is widely distributed in southern Mexico. It has been used in traditional herbal medicine because of its potential health beneficial properties related to phenolic compounds, among them, flavonoids have been known to be the nature's tender drugs to show a wide range of pharmacological activities [1]. They are a group of chemical entities of benzo-γ-pyrone derivatives that have been studied as potential anti-inflammatory reagents [2]. Flavonoids can affect the functions of cells linked to inflammatory processes, acting on enzymes and pathways involved in anti-inflammatory processes [3]. Previous chemical analyses of *E. platycarpa* have allowed the isolation of flavonoids as 5,7-dihydroxy-6-methyl-8-prenylflavanone; 5,7-dihydroxy-6-methyl-8-prenyl-4′-methoxy-flavanone; 5,7-dihydroxy-6-prenylflavanone and 5-hydroxy-7-methoxy-6-prenylflavanone [4]. Thus, it may be valuable to study

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http://dx.doi.org/10.1016/j.colsurfb.2013.12.009

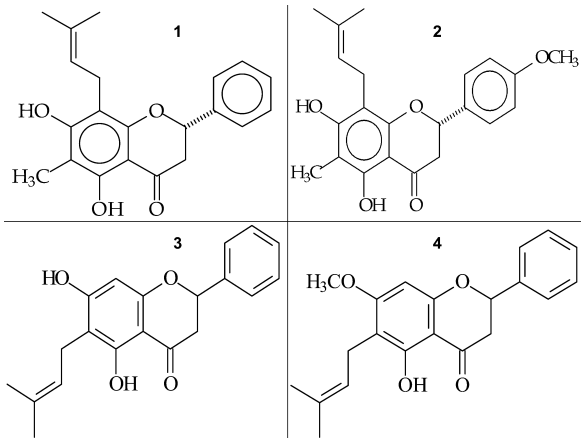

Fig. 1. Chemical structure of flavanones. 5,7-Dihydroxy-6-methyl-8-prenylflavanone (1); 5,7-dihydroxy-6-methyl-8-prenyl-4′-methoxy-flavanone (2); 5,7-dihydroxy-6-prenylflavanone (3); and 5-hydroxy-7-methoxy-6-prenylflavanone (4).

the anti-inflammatory activity of these flavonoids, not only in order to establish anti-inflammatory mechanisms, but also for developing a new class of safe anti-inflammatory agents, which may be useful in the treatment of these kinds of diseases [5].

However, the chemical structure of flavonoids (Fig. 1) makes them water insoluble compounds. To overcome the poor water solubility of drugs, an important area of pharmaceutical research is in finding safe and effective strategies to deliver conventional drugs as nano-scale drug delivery systems which can be devised to tune release kinetics, to regulate biodistribution and to minimize side effects, thereby enhancing the therapeutic index of a given drug [6]. Nanoemulsions and nanoparticles are being widely utilized in pharmaceutical and biomedical sciences [7–10]. In fact, many promising new or existing drugs never reach the market because of difficulties in delivery. Such drugs need to be formulated with smart drug delivery systems and/or delivery technology to make them acceptable for the treatment of patients [11]. Simultaneously, drug delivery systems can be synthesized with controlled composition, shape, size and morphology.

Nanoemulsions are transparent or translucent dispersions of oil and water stabilized by an interfacial film of surfactant usually in combination with co-surfactant having droplet sizes less than 100 nm. From the pharmaceutical point of view, nanoemulsions improve transdermal and dermal drug delivery *in vitro* and *in vivo* over classical dosage forms, as well as, increase the aqueous solubility of poorly water soluble drugs as in the case of the referred flavanones [12].

Nanoparticle systems have also been proposed for topical administration to enhance percutaneous transport into and across the skin barrier with important features, among them their surface to mass ratio that is much larger than other particles, their quantum properties and their ability to bind and adsorb drugs improving their bioavailability, providing better encapsulation, controlled release, less toxicity and increasing drug stability [8]. In this line, upon skin application, the stability of polymeric carriers differs considerably from conventional vesicles (liposomes and niosomes). [13]

In view of an extensive literature searching, no information about *E. platycarpa* was found as anti-inflammatory agent loaded nanostructured drug delivery systems for dermal application and on account of alleged usefulness to this plant in traditional medicine, the major aims of the present study were: (i) to develop nanoemulsion (NE) and nanoparticles (NP) of flavanones: 5,7-dihydroxy-6-methyl-8-prenylflavanone (**1**); 5,7-dihydroxy-6-methyl-8-prenyl-4′-methoxy-flavanone (**2**); 5,7-dihydroxy-6-prenylflavanone (**3**) and 5-hydroxy-7-methoxy-6-prenylflavanone (**4**) isolated from the methanolic extract of *E. platycarpa* leaves, (ii) to characterize the formulations in terms of physicochemical properties; (iii) to evaluate their *in vitro* release behaviour and kinetic parameters, (iv) to investigate the *ex vivo* permeation across human skin and retained amounts, and finally (v) to assess the *in vivo* anti-inflammatory activity in mice.

2. Materials and methods

2.1. Chemicals

Poly lactic-co-glycolic acid (PLGA) 50:50 (Resomer® RG503) was obtained from Boehringer Ingelheim (Ingelheim, Germany), poloxamer 188 (P188, Lutrol® F68) was kindly provided by BASF Corp. (Barcelona, Spain), polyglyceryl-3 dioleate (Plurol® Oleique CC497), caprylocaproyl polyoxyl-8-glycerides (Labrasol®), triglycerides medium-chain EP/NF/JPE (Labrafac® lipophile) and propylene glycol were provided by Gattefossé (Saint-Priest Cedex, France). Double distilled water was used after filtration in a Millipore® system (Millipore, Billerica, MA, USA). All other chemicals and reagents used in the study were of analytical grade and obtained from Panreac (Barcelona, Spain).

2.2. Experimental animals

Male Swiss CD-1 mice (20–25 g) were purchased from Circulo ADN S.A. de C.V. (Coyoacan D.F., Mexico) and were subjected to a quarantine period of 7 days on arrival. The animals were housed in plastic cages with soft bedding with access to controlled diet and tap water *ad libitum*. The temperature was kept at 24 ± 1 °C and the relative humidity was kept at 50–60%. Artificial lighting was used to provide 12 h light and 12 h dark every 24 h. The studies were conducted under a protocol in accordance with the Mexican Official Norm for Animal Care and Handling (NOM-062-ZOO-1999).

2.3. Extraction and isolation

E. platycarpa leaves were collected from the municipality of Tetipac, State of Guerrero (México) and identified by Prof. Ramiro Cruz. A voucher specimen was stored at the Sciences Faculty herbarium facilities of the Autonomous University of the State of Morelos, Mexico (voucher specimen Ramiro Cruz 1325).

Once the leaves were dried at room temperature they were pulverized and extracted with methanol by maceration at room temperature three times (100 g of dried vegetable material per 1000 mL of methanol).The extract was concentrated to dryness by rotatory vaporization at 60 °C under reduced pressure. The prenylated flavanones **1**, **2**, **3** and **4**, were isolated by column chromatography at reduced pressure, and then flavanones were purified and characterized by direct comparison using TLC original samples available at the laboratory. They were also analyzed by ^{1}H NMR and ^{13}C NMR and mass spectrometry checking their identities by comparison with the spectroscopic available data in literature [4].

Finally, the logarithm of the molecular octanol/water partition coefficient (log *P*) of the isolated flavanones was estimated, because is a measure of hydrophobicity, an important property to predict or explain the drug behaviour. The measure was performed by the semiempirical quantum AM1 method using the HyperChem Professional 8.0 software (Hypercube, Inc., Gainesville, FL, USA).

2.4. Preparation of nanoparticulate systems

The elaboration of flavanones loaded NEs (NE1, NE2, NE3, NE4) was performed according to the method described previously [9] by mixing the established amounts of oil, solvent