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Phyto-mediated nanostructured carriers based on dual vegetable actives involved in the prevention of cellular damage

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ABSTRACT

The growing scientific interest in exploitation of vegetable bioactives has raised a number of questions regarding their imminent presence in pharmaceutical formulations. This study intends to demonstrate that a dual combination between vegetable oil (*e.g. thistle oil*, *safflower oil*, *sea buckthorn oil*) and a carrot extract represents an optimal approach to formulate safe carrier systems that manifest cell regeneration effect and promising antioxidant and anti-inflammatory activity. Inclusion of both natural actives into lipid carriers imparted a strong negative charge on the nanocarrier surface (up to −45 mV) and displayed average sizes of 70 nm to 140 nm. The entrapment efficiency of carrot extract into nanostructured carriers ranged between 78.3 and 88.3%. The *in vitro* release study has demonstrated that the entrapment of the extract represents a viable way for an equilibrated release of carotenoids. Besides the excellent antioxidant properties (*e.g.* scavenging up to 98% of the free oxygen radicals), the results of cellular integrity (*e.g.* cell viability of 133%) recommend these nanocarriers based on dual carrot extract–bioactive oil as a promising trend for the treatment of certain disorders in which oxidative stress plays a prominent role. In addition, the lipid nanocarriers based on *safflower oil* and *sea buckthorn oil* demonstrated an anti-inflammatory effect on LPS induced THP-1 macrophages, by inhibiting the secretion of two pro-inflammatory cytokines, IL-6 and TNF-α.

1. Introduction

The use of various vegetable entities has received considerable attention in nano-biotechnology and nano-medicine fields owing to the huge potential of many plant derived compounds, *e.g.* vegetable oils and extracts, to have curative potentials and to manifest multiple nutritional and therapeutical properties [1,2]. World Health Organization estimated that approximately 80% of world's inhabitants rely mainly on the traditional medicines for their primary health care and at least 119 chemical substances derived from 90 plant species can be considered as important drugs [3]. Among the plant-derived compounds, natural antioxidants have gained a great interest due to their effectiveness in preventing and combating health problems caused by oxidative stress [4]. Reactive oxygen species (ROS) have been involved in the causation and progression of several chronic diseases, including cancer and cardiovascular diseases, the two major causes of morbidity and mortality in Western world [5]. Dietary antioxidants, which inactivate ROS and provide protection against oxidative damage, are considered as important preventive strategic molecules [6]. Carotenoids are organic pigments naturally synthesized by plants that have been implicated as dietary nutrients in the scavenging of ROS, *e.g.* singlet molecular oxygen and peroxyl radicals generated in the process of lipid peroxidation [7]. However, the effective utilization of these lipophilic carotenoids is limited by their low bioavailability, poor permeability, and/or precarious stability in the gastro-intestinal environment [8]. Several studies demonstrated that the carotenoids are more bioavailable when they are solubilized in oil carriers compared to the case when they are present as crystals in vegetable tissues, due to an easier transfer to the mixed micelles that can transport them to the epithelium cells for absorption [9,10].

The encapsulation of highly lipophilic carotenoids and other functional plant mixtures using nanotechnology approaches could address several concerns, *e.g.* improve their bioavailability, prevent the degradation of labile compounds, enhance the biological activity or control release of the entrapped ingredients [11,12]. For instance, nanoencapsulation of hydrophobic *Guabiroba extract* into poly(lactide-*co*-glycolide) nanoparticles was achieved for antimicrobial and antioxidant delivery applications [13]. The enhancement of skin-protective activities of w/o-type spherical liposomes encapsulating *Centella asiatica extract* [14], the improvement of the stability and antioxidant activity of *Jujube seed extracts* by loading into chitosan nanoparticles [15], the enhancement of the antibacterial activity of silver nanoparticles based on *Cassia tora leaves extract* [16] or the nanoencapsulation of *Saffron extract* through w/o/w multiple emulsions to increase the stability of extract [17] are several studies developed in the last two years that

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Table 1
Fatty acids content of vegetable oils quantified by gas chromatography.

Vegetable oil	Linolenic acid (ω−3), %	Linoleic acid (ω−6), %	Palmitoleic acid (ω−7), %	Oleic acid (ω−9), %	Other/saturated fatty acids, %
Thistle oil (TO)	4.09	44.37	–	29.19	22.35
Safflower oil (SO)	0.13	77.64	–	11.60	10.63
Sea buckthorn oil (SBO)	2.00	3.60	30.10	21.90	33.64

demonstrate the benefices of these natural compounds. Beside these systems, the nanostructured lipid carriers (NLC) made by biocompatible lipids and surfactants can be used as efficient delivery vehicles due to their low toxicity, great stability, being cost effective for industrial production in pharmaceutical, cosmetic and food applications [18,19]. These nanocarriers have been used for nearly two decades for nanoencapsulation of synthetic drugs, but only recently the scientists have begun to utilize them for entrapping plant derived compounds, *e.g.* eugenol [20], curcumin [21], silymarin [22], bixin [23] and quercetin among others [24] in order to increase their bio-pharmaceutical qualities.

On the other hand, the carrot extract represents a valuable source of phytochemicals with a high nutritional and medicinal value that can modulate the reactive oxygen species (ROS) level and that exhibits a unique profile of carotenoids such as carotene and lutein, with special role in health promotion and prevention of coronary heart diseases, obesity, hypertension and inflammatory diseases [25,26]. Aside from being an important source of provitamin A, the functional activities of carrot extract continue to be discovered, such us prevention of osteoporosis [27], skin protective effects [28], as well as anticancer potential [29].

As far as we know, there is no available scientific literature about nanoencapsulation of vegetable extract in association with medicinal plant oils *via* nanostructured lipid carriers (NLC). With this evidence, the present study aimed at formulating unique phyto-mediated nanostructured carriers able to increase the bioavailability of highly lipophilic carrot extract by its solubilization in appropriate vegetable oils. Here we demonstrate the direct connection between the ability of nanostructured carriers based on dual carrot extract–bioactive vegetable oil to quench free radicals and to preserve the cell integrity, by means an increased cell regeneration effect. A series of medicinal plant oils extracted from Romanian herb seeds (*e.g. thistle oil*, *safflower oil*, *sea buckthorn oil*) was investigated as appropriate oily medium for lipophilic carrot extract solubilization and for nanostructured carriers synthesis. The lipid profiles of the selected vegetable oils show different concentrations of ω−6 fatty acids, *e.g.* 44.6% in *thistle oil*, 77.6% in *safflower oil* and 3.6% in *sea buckthorn oil*. Besides the constitutive purpose of the vegetable oil (being a main component of the nanostructured vehicle), ω−6 fatty acids have a therapeutic purpose, which will be enhanced by encapsulation at nanoscale. These phyto-mediated nanocarriers may provide a new opportunity to encourage the use of targeted delivery systems with high content in vegetable oil for encapsulation and delivery of other lipophilic extracts.

2. Experimental

2.1. Materials

Synperonic PE/F68, L-α-phosphatidylcholine and polyoxyethylenesorbitan monolaurate (Tween 20) were acquired from Sigma Aldrich Chemie GmbH (Germany) and Merck (Germany), respectively. Beeswax (BW) was provided by Apimondia S.R.L., Romania; glycerol monostearate (GM) and cetyl alcohol (CA) were obtained from Cognis GmbH (Germany) and Acros Organics (USA). Other materials employed were tris[hydroxymethyl] aminomethane, 5-amino-2,3-dihydro-1,4-phthalazinedione (luminol) (from Sigma Aldrich Chemie GmbH) and hydrogen peroxide (from Merck, Germany).

A mouse fibroblast cell line (NCTC clone L929) was purchased from ECACC (Sigma-Aldrich, Germany), a human monocyte leukemia THP-1 cell line was purchased from American Type Culture Collection (Manassas, VA, USA). Minimum Essential Medium (MEM), RPMI 1640 medium, fetal bovine serum (FBS), PSN (penicillin, streptomycin, neomycin), 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT), isopropanol, phorbol 12-myristate 13-acetate (PMA) and lipopolysaccharides from *Escherichia coli* (LPS) were purchased from Sigma-Aldrich Chemicals (Germany).

The carrot oily extracts (CE) were provided by Hofigal S.A. Company (Romania) and were obtained from carrot roots by solvent extraction technique followed by concentration of obtained extracts in three kinds of vegetable oils – *thistle oil*, *safflower oil* and *sea buckthorn oil*. The total carotenoids concentrations (expressed in β-carotene) determined by UV–VIS Spectroscopy (UV–VIS Spectrophotometer Cintra 6, $\lambda = 460$ nm) were of 187 mg carotenoids/100 g *thistle* oily extract, 147 mg carotenoids/100 g *safflower* oily extract, and 350 mg carotenoids/100 g *sea buckthorn* oily extract, respectively. The vegetable oils (*thistle oil*, *safflower oil* and *sea buckthorn oil*) were obtained by cold pressed method from milk thistle, safflower seeds and sea buckthorn fruits and have been analyzed by gas chromatography in order to determine the fatty acids content. The composition of each vegetable oil was determined by gas-chromatography (Gas Chromatograph Thermo Electron Corporation Focus), HP-5MS column, He carrier gas (flow rate of 1 mL/min), injection sample volume of 1.0 μL. Detection was performed using the Mass Spectrometer (Thermo Electron Corporation-DSQII). The main fatty acids determined in the selected vegetable oil were detailed in Table 1.

2.2. Synthesis

Various nanostructured lipid carriers were prepared according to the compositions described in Table 2 by high pressure homogenization technique. The conditions used for the preparation of lipid nanocarriers containing different concentrations of carrot extract were adapted from previous works of authors [30,31]. Briefly, several pre-emulsions were obtained by the dispersion of a melted lipid phase (consisting in 8% mixture of GM, CA, BW and vegetable oils – TO, SO and SBO) in an emulsifier hot solution (consisting of distilled water and 2.5% mixture of Tween 20:phosphatidylcholine:block copolymer). The pre-emulsions have been kept for 20 min at 80 °C and were firstly subjected to a high shear homogenization stage (High-Shear Homogenizer PRO250 type; 0–28.000 rpm; power of 300 W, Germany), by applying 12,000 rpm for 1 min and then to a high pressure homogenization (APV 2000 Lab Homogenizer, Germany), at 500 bar for 196 s. The aqueous nanodispersions were cooled at room temperature to obtain the nanostructured carriers. The composition of blank and loaded-NLC with carrot extract is detailed in Table 2. The aqueous dispersions of NLC were frozen at −25 °C overnight and were lyophilized for 72 h, at −55 °C using an Alpha 1–2 LD Freeze Drying System Germany.

2.3. Characterization of nanostructured carriers by entrapping dual vegetable actives

2.3.1. Dynamic light scattering

Particle size of the aqueous NLC dispersions was determined by dynamic light scattering (DLS), yielding the mean particle size (Z_{ave}) and