European Journal of Pharmaceutical Sciences 97 (2017) 62–69

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

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

Combination of grape extract-silver nanoparticles and liposomes: A totally green approach

Ines Castangia [a], Francesca Marongiu [a], Maria Letizia Manca [a], Raffaello Pompei [b], Fabrizio Angius [b], Andrea Ardu [c,d], Anna Maria Fadda [a], Maria Manconi [a,*], Guido Ennas [c]

[a] Dipartimento Scienze della Vita e dell'Ambiente, Università di Cagliari, Cagliari, Italy
[b] Dipartimento Scienze Biomediche, Università di Cagliari, Cagliari, Italy
[c] Dipartimento Scienze Chimiche e Geologiche, Università di Cagliari e Unità di Ricerca INSTM di Cagliari – Cittadella Universitaria di Monserrato, 09042, Monserrato, (CA), Italy
[d] Consorzio AUSI, Palazzo Bellavista Monteponi, Iglesias, Carbonia-Iglesias, Italy

ARTICLE INFO

Article history:
Received 2 September 2016
Received in revised form 28 October 2016
Accepted 2 November 2016
Available online 4 November 2016

Keywords:
Grapes pomace
Phospholipids vesicles
Keratinocytes
Fibroblasts
Oxidative stress
Staphylococcus aureus

ABSTRACT

In the present work, silver nanoparticles were prepared using a totally green procedure combining silver nitrate and an extract of grape pomace as a green source. Additionally, nanoparticles were stabilized using phospholipid and water and/or a mixture of water and propylene glycol (PG). To the best of our knowledge, grape-silver nanoparticle stabilized liposomes or PG-liposomes were formulated, for the first time, combining the residual products of wine-made industry, silver nitrate and phospholipids, avoiding the addition of hazardous substances to human health and the environment, in an easy, scalable and reproducible method. The structure and morphology of grape-silver nanoparticle stabilized vesicles were evaluated by transmission electron microscopy (TEM), UV-vis spectroscopy and photon correlation spectroscopy. Samples were designed as possible carrier for skin protection because of their double function: the grape extract acts as antioxidant and the colloidal silver as antimicrobial agent, which might be helpful in eliminating dangerous free radicals and many pathogenic microorganisms. Obtained nanoparticles were small in size and their combination with phospholipids did not hamper the vesicle formation, which were multilamellar and sized ~100 nm. TEM images shows a heterogeneous distribution of nanoparticles, which were located both in the intervesicular medium and in the vesicular structure. Further, grape-silver nanoparticles, when stabilized by liposomes, were able to inhibit the proliferation of both *Staphylococcus aureus* and *Pseudomonas aeruginosa* and provided a great protection of keratinocytes and fibroblasts against oxidative stress avoiding their damage and death.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Properties of silver have been known since ancient times and it has been successfully used in the medical field for antimicrobial applications and even in the treatment of open wounds and burns, before the introduction of antibiotic therapy, thanks to its antimicrobial properties (Hidalgo, 1998; Sharma et al., 2009; Sondi and Salopek-Sondi, 2004). More recently, silver nanoparticles have been introduced as innovative alternative to silver solution and tested in a broad range of applications such as drug delivery, diagnostic medicine, food safety, disinfection, and water treatment (Regiel et al., 2013). These nanoparticles are able to improve the antimicrobial activity of the metal thanks to their ability to control its gradual oxidation and release as ionic silver in biological media (Xiu et al., 2012). Furthermore, silver nanoparticle may prevent its inactivation, which easily occurs by complexation and precipitation, being a valid alternative to ionic silver solutions (Rai et al., 2009). Colloidal silver with specific sizes and morphologies can be prepared using different chemical and physical methods (Campi et al., 2010; Li et al., 2007; Mari et al., 2010). The chemical approach is the most widely used despite its complexity, high costs and the use of hazardous substances to human health and the environment such as reducing agents (dimethyl formamide, hydrazine or aniline) and stabilizer agent (e.g. polyvinylpyrrolidone), the latter mainly used to control particle size, prevent aggregation and subsequent sedimentation (Giallongo et al., 2013; Wei et al., 2009).

The bioproduction of nanoparticles has been proposed as a friendly alternative to chemical and physical methods, which involves the choice of a green solvent medium, an environmentally benign reducing agent (natural biological molecules or microorganism), and a nontoxic material for their stabilization. (Raveendran et al., 2003; Regiel et al., 2013; Vigneshwaran et al., 2007). Using cheap and safe compounds, the preparation of silver nanoparticles becomes less expensive and impacting, allowing them to be used in new application fields as colloidal and non-toxic materials. In the last decade, phytofabrication of metallic nanoparticles for pharmaceutical applications has attracted a great

* Corresponding author.
E-mail address: manconi@unica.it (M. Manconi).

http://dx.doi.org/10.1016/j.ejps.2016.11.006
0928-0987/© 2016 Elsevier B.V. All rights reserved.

attention and the suitable use of silver nanoparticles reduced by herbal extracts has opened new perspectives for their potential phytotherapeutic uses as antioxidant and antimicrobial agents (Bar et al., 2009; Bhuvaneswari and Thiyagarajan, 2014; Jeeva and Thiyagarajan, 2014; Kavitha et al., 2013; Kotakadi et al., 2014).

Grape is one of the world's largest fruit crop, and its beneficial effects have been extensively investigated for its high content in polyphenols and consequent strong antioxidant ability (Giovinazzo and Grieco, 2015). A great part of grape world production is aimed at wine making with an important accumulation of grape pomace, which is considered a wine-by-product, difficult to eliminate (Yu and Ahmedna, 2013). A large amount of grape polyphenols still remains in pomace because seeds and skins are rich in them, and, as a consequence, the pomace extract may represent an important source of natural and inexpensive antioxidants and can be used as reducing agent during metal nanoparticle preparation (Xu et al., 2014). Obtained silver nanoparticles are generally characterized by high surface energy, which makes them extremely reactive causing their rapid aggregation, if no protection or passivation of their surfaces is performed (Raveendran et al., 2003). In this work silver nanoparticles were, for the first time, simultaneously synthetized and stabilized, in a unique step, by using phospholipid vesicles. A new, one-step, green-fabrication method was optimized thus obtaining grape extract-silver nanoparticle stabilized liposomes as suitable topical antimicrobial and antioxidant formulation, considering the well-known performances of phospholipid nanovesicles as skin delivery systems. Thanks to their versatility, vesicles can simultaneously load the different components of the extract in their hydrophilic and hydrophobic compartments and stabilize the metal nanoparticles avoiding their aggregation and precipitation (Floris et al., 2011). Liposomes were also chosen to increase components stability and target all the therapeutic agents to specific tissues and cells (Zhao et al., 2013). Liposome performances can be improve using different water co-solvents and in previous works propylene glycol (PG) showed promising abilities as skin carriers (Castangia et al., 2013; Manconi et al., 2009), probably because this solvent can act as penetration enhancer like to ethanol and simultaneously can modulate the elasticity of vesicle bilayer (Zhang et al., 2012). In order to this, in the present work, PG-liposomes were used alternatively to basic liposomes to stabilize and deliver grape-silver nanoparticles. The physico-chemical characteristics of resulting vesicles, such as morphology, size and zeta potential, were evaluated. The antibacterial as well as the antifungal activity, against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*, were studied in vitro. Finally, the in vitro toxicity, and the aptitude of nanocarriers to protect the cells from oxidative stress damage in human keratinocytes and 3T3 mouse fibroblasts were assessed.

2. Materials and methods

2.1. Materials

Soy phosphatidylcholine (SL) was purchased from Galeno S.r.l. (Milan, Italy). Silver nitrate, propylene glycol (PG), quercetin, gallic acid, dimethyl sulfoxide and all the other products were purchased from Sigma-Aldrich (Milan, Italy). Dulbecco's Modified Eagle's medium, RPMI1640 medium, glucose, foetal bovine serum, penicillin, and streptomycin were purchased from Life Technologies Europe (Monza, Italy). Fresh pomace of Cannonau, was provided by Vini Gostolai S.r.l. (Oliena, Italy).

2.2. Grape extract preparation

Aliquots of wet pomace were weighed (100 g), mixed with 900 ml of ethanol/water blend (1/1w/w), homogenised and maintained under constant stirring for 48 h. At the end of the extraction procedure, the dispersions were centrifuged two times (30 min, 8000 rpm), the supernatants were collected and evaporated under vacuum at 30 °C to eliminate the ethanol. Finally, samples were lyophilized and a violet-brown hygroscopic powder was obtained.

2.3. Folin-Ciocalteu method

The total phenolic content was measured according to the Folin-Ciocalteu colorimetric assay using a UV spectrophotometer (Lambda 25, Perkin Elmer, Monza, Italy). Extract was dissolved in ethanol (1 mg/ml) and, 100 μl of the extract solution, 100 μl of the Folin-Ciocalteu reagent and 800 μl of 20% (w/v) Na_2CO_3 water solution were mixed together and the absorbance was read at 765 nm after 30 min of incubation at room temperature in the dark. The total phenolic content was calculated by means of a calibration curve built using gallic acid as a reference, at different concentrations (0–0.125 mg/ml). Results, expressed as mg of gallic acid equivalents per g of dried extract (EGA), were the means of six determinations (Castangia et al., 2015).

2.4. In vitro DPPH assay

The antioxidant activity of the grape extract was evaluated by measuring their ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH accepts a hydrogen atom from the scavenger molecule (i.e. antioxidants) reducing itself, changing the colour from purple to yellow and decreasing its absorbance intensity at 517 nm. Each sample was diluted (1:100) with the DPPH methanolic solution (40 μg/ml). The same dilution (1:100) of DPPH methanolic solution was used as a control. All the samples were stored at room temperature for 30 min in the dark. Then, the absorbance was measured at 517 nm against blank. The percent antioxidant activity was calculated according to the following formula (Manca et al., 2015a, 2015b):

$$\text{antioxidant activity } (\%) = [(ABS_{DPPH} - ABS_{sample})/ABS_{DPPH}] \times 100$$

The efficient concentration (EC50), which represent the sample concentration (μg/ml) required to decrease (50%) the initial DPPH absorbance, and the antiradical power (ARP) namely the inverse of EC50 value (ARP = 1/EC50) were calculated. ARP quantifies the antioxidant activity of samples and high values correspond to a greater antioxidant activity. Results were expressed as the means of six determinations.

2.5. Synthesis and characterization of grape-silver nanoparticles

Grape extract (20 mg/ml) and silver nitrate (10 mM) were dispersed in water. The mixture was sonicated using a high intensity ultrasonic disintegrator (Soniprep 150, MSE Crowley, London, United Kingdom) for 10 cycles (2 s on and 5 s off, 13 microns of probe amplitude) with pause after each cycle to promote the cooling of the samples.

The synthesis of silver nanoparticles was firstly evidenced by the colour of dispersion which changed from pale red to dark brown accompanied by a mirror like illumination on the flask due to the reduction of silver ions to metallic silver. The UV-vis absorption spectrum of nanoparticles was recorded using a Spectrometer (Lamba 25, Perkin Elmer, Monza, Italy) by scanning the sample at a wavelength ranged between 200 and 800 nm, using a resolution of 1 nm slit width and 0.3 nm/scan rate.

To observe the samples by high resolution transmission electron microscopy (HRTEM), the silver suspension was directly dropped on carbon-coated copper grids and the excess was removed with filter paper. Samples were observed using a microscope, JEOL JEM 2010 UHR equipped with a Gatan Imaging Filter, with a 15 eV window and a 794 slow scan CCD camera, operating at 200 kV.

2.6. Preparation of grape-silver nanoparticle stabilized liposomes

Silver nitrate (10 mM) and the grape extract (20 mg/ml) were dispersed in water or propylene glycol/water (10% w/v) solution. The obtained dispersions were added to phospholipids (SL, 120 mg/ml) to prepare liposomes or PG-liposomes. Phospholipid dispersions were

Download English Version:

https://daneshyari.com/en/article/5547978

Download Persian Version:

https://daneshyari.com/article/5547978

Daneshyari.com