Colloids and Surfaces B: Biointerfaces 121 (2014) 299–306

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Bio-active nanoemulsions enriched with gold nanoparticle, marigold extracts and lipoic acid: In vitro investigations

Emine Guler[a], F. Baris Barlas[a], Murat Yavuz[a,b], Bilal Demir[a], Z. Pinar Gumus[c], Yucel Baspinar[d], Hakan Coskunol[c,e,f], Suna Timur[a,c,*]

[a] Ege University, Faculty of Science, Biochemistry Department, 35100 Bornova, Izmir, Turkey
[b] Dicle University, Faculty of Science, Chemistry Department, 21280 Diyarbakir, Turkey
[c] Ege University, Institute of Drug Abuse Toxicology & Pharmaceutical Sciences, 35100 Bornova, Izmir, Turkey
[d] Ege University, Faculty of Pharmacy, Department of Pharmaceutical Biotechnology, 35100 Bornova, Izmir, Turkey
[e] Ege LS, Ege University, 35100 Bornova, Izmir, Turkey
[f] Ege University, School of Medicine, Department of Psychiatry, 35100 Bornova, Izmir, Turkey

ARTICLE INFO

Article history:
Received 10 March 2014
Received in revised form 27 April 2014
Accepted 16 May 2014
Available online 23 June 2014

Keywords:
Nigella sativa oil
Calendula officinalis extract
Gold nanoparticle
Lipoic acid
Herbal nanoemulsions

ABSTRACT

A novel and efficient approach for the preparation of enriched herbal formulations was described and their potential applications including wound healing and antioxidant activity (cell based and cell free) were investigated via in vitro cell culture studies. *Nigella sativa* oil was enriched with *Calendula officinalis* extract and lipoic acid capped gold nanoparticles (AuNP–LA) using nanoemulsion systems. The combination of these bio-active compounds was used to design oil in water (O/W) and water in oil (W/O) emulsions. The resulted emulsions were characterized by particle size measurements. The phenolic content of each nanoemulsion was examined by using both colorimetric assay and chromatographic analyses. Two different methods containing cell free chemical assay (1-diphenyl-2-picrylhydrazyl method) and cell based antioxidant activity test were used to evaluate the antioxidant capacities. In order to investigate the bio-activities of the herbal formulations, in vitro cell culture experiments, including cytotoxicity, scratch assay, antioxidant activity and cell proliferation were carried out using Vero cell line as a model cell line. Furthermore, to monitor localization of the nanoemulsions after application of the cell culture, the cell images were monitored via fluorescence microscope after FITC labeling. All data confirmed that the enriched *N. sativa* formulations exhibited better antioxidant and wound healing activity than *N. sativa* emulsion without any enrichment. In conclusion, the incorporation of AuNP–LA and *C. officinalis* extract into the *N. sativa* emulsions significantly increased the bio-activities. The present work may support further studies about using the other bio-active agents for the enrichment of herbal preparations to strengthen their activities.

1. Introduction

Natural preparations from herbal sources have been used to fight against diseases since ancient time [1–7]. There is a great interest for the use of plant extracts or herbal oils due to their beneficial effects on human health [8]. In recent years, there is a considerable growth in natural product industries containing not only food and beverages, but also pharmaceuticals and cosmetics [9]. Taking into consideration all of these wide knowledge and background for natural preparations, investigations about the potential use of plant materials for medical and cosmetic purposes are becoming more and more important.

Nigella sativa, known as black cumin, belongs to the Ranunculaceae family. This annual plant has been used traditionally for the therapy of several diseases. *N. sativa* seed oil has a wide range of therapeutic effects such as anti-inflammatory [10], antitumor [11], anti-ulcer [12], antimicrobial [13], antiasthmatic [14], antifungal [15], and analgesic effects [16]. Chemical constituents and biological activities of *N. sativa* have been widely investigated and many components containing also active ingredients have been identified successfully [17–19]. Thymoquinone (TQ) which is an active constituent of *N. sativa* has a great deal of attention because of its important role in pharmaceutical processes. A large number of studies reported that *N. sativa* and TQ

* Corresponding author at: Ege University, Faculty of Science, Biochemistry Department, 35100 Bornova, Izmir, Turkey. Tel.: +90 232 311 2455; fax: +90 232 311 5485.
E-mail addresses: suna.timur@ege.edu.tr, sunatimur@yahoo.com (S. Timur).

http://dx.doi.org/10.1016/j.colsurfb.2014.05.026
0927-7765/

have an antioxidant effect via powerful radical scavenging activity [20–23]. Antioxidants from natural sources such as medicinal plants have some advantages compared to synthetic antioxidants e.g. butylatedhydroxyanisole (BHA) and butylatedhydroxyltoluene (BHT). Natural antioxidants are more effective and less toxic than others of non-natural origin, which are suspicious about their carcinogenic effect and damaging effect to the liver [24]. Reactive oxygen species (ROS) which are formed under physiological conditions such as UV radiation, inflammation, cancer, and diabetes are generally harmful for body related to the oxidative stress. ROS are playing critical role in various processes such as wound healing and aging. Antioxidant systems provide the reduction of oxidative damage via radical species formation [25]. Hence, both the investigation of antioxidants from natural sources and enrichment of them with other bioactive materials to increase the efficiency are getting significant for the cosmetic and the health sector.

Calendula officinalis (Asteraceae), termed as Marigold, has been used in folk medicine for the treatment of problems such as skin wounds, cuts, burns and other dermatological diseases [26]. It has a significant historical background owing to its therapeutic potential containing anti-inflammatory, antioxidant, and wound healing, antibacterial, antifungal and antiviral effects [27]. Calendula plants are officially involved in European Pharmacopeia [28] and also its flowers or extracts have been used for different commercial preparations like ointments, creams, tea, spice, cosmetic cream [29] and a large number of personal care products. The topical use of Calendula preparations with the purpose of cosmetic and personal care product like skin protection agent was recognized to be safe at proper concentrations by UNITIS, the European Organization of Cosmetic Ingredients Industries and Services [30].

Gold nanoparticles (AuNPs) have been considerably used for the biomedical applications such as diagnostic, delivery systems for some molecules like a drug, protein, gene, and lipid, for the therapy of some angiogenesis and inflammatory based diseases and photodynamic therapy [31–33]. The most important point for the use of AuNPs in the field of nano-medicine is the fact that they are not-cytotoxic and not-immunogenic [34]. Furthermore, they can be produced easily with the desired particle size. One of the crucial advantages is their easy functionalization and conjugation. AuNPs can simply conjugate with targeting and therapy agents via covalent and non-covalent attachments [35,36]. In a previously published work, it was reported that AuNPs can increase the proliferation of keratinocyte cells subject to the size and concentration of nanoparticles [37]. In another study, it was described that AuNPs enhanced the healing of skin wound in mice due to its anti-inflammatory and antioxidative effects [38]. In the recent experiments, it was described that AuNPs can pass through the skin barrier via opening the stratum corneum [39]. Hence, the use of antioxidative and wound healing agents with AuNPs can be promising for the development of new effective products like topical skin protectors thanks to their synergistic effect and increased absorption to the skin.

α-Lipoic acid (LA) has been known as a universal antioxidant agent for decades. LA is naturally present in human and other organisms as a cofactor for mitochondrial enzymes, therefore it plays a key role in mitochondrial energy pathways. In a great number of studies, it was described that both LA and its reduced form, dihydrolipoic acid (DHLA) have a strong ROS scavenging ability and a metal-chelating activity [40–42]. According to clinical and experimental studies, it is reported that LA is a potential therapeutic source of some health problems like diabetes, heavy metal intoxication, radiation damage, aging and other oxidative stress related diseases [43]. Commercial products of LA are used as dietary supplement due to its high pharmacological value [44–46].

Under the light of the above mentioned information, *N. sativa* oil and its enriched formulations with *C. officinalis* extract and/or LA capped AuNP were prepared in this present work. The aim was to investigate the bio-activities of the preparations and to determine the most-effective formulation. It has been known that the novel compounds obtained by combining two and more biologically active compounds could demonstrate the enhanced bio-activities [47–51]. Therefore, we hypothesized that combinations of *N. sativa* oil, *C. officinalis* extract and AuNP–LA could show a stronger biological effect. In that context, we intend to obtain more information about enriched herbal preparations and to investigate their potential uses as health supports with their proliferative effects as well as wound healing properties.

2. Materials and methods

2.1. Materials

AuNPs (40 nm) were purchased from BBI Solutions (Cardiff, UK). Black seed (*N. sativa*) oil obtained by the CO_2 extraction method was kindly donated from TABIA (Turkey). Folin-Ciocalteu reagent, gallic acid (3,4,5-trihydroxybenzoic acid), phosphate buffered saline (PBS, pH 7.4), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), sodium dodecyl sulfate (SDS) were purchased from Sigma. Sodium carbonate (Na_2CO_3) was obtained from Merck. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Aldrich. L-α-Phosphatidylcholine from egg yolk, Tween 85, caffeic acid, vanilic acid, chlorogenic acid, p-coumaric acid, trans-2-hydroxycinnamic acid, thymoquinone (TQ), methanol (HPLC grade), ethanol, hexane, methyl tert-butyl ether, 2-propanol and diamino-2-phenylindol (DAPI) were purchased from Sigma–Aldrich. Water was purified in a Milli-Q plus System (Millipore).

C. officinalis extract was prepared according to a solvent extraction method using methanol [52]. After the solvent was evaporated, the dried extract was used for the preparation of the emulsions by dissolving it with ethanol:distilled water (5:1).

2.2. Conjugation of LA to AuNPs

Disulfide bond which is found in the structure of LA was reduced with dithiothreitol (DTT) to generate the —SH groups according to the method described previously [53]. To conjugate AuNP with LA, we use the strong affinity of AuNPs to sulfur atoms. Thus, 0.06 mg/mL AuNPs (40 nm in size) were incubated with the reduced LA (10 mg/mL) in ethanol solution for 12 h at +4 °C under agitation at 750 rpm. After that AuNPs were washed with distilled water via centrifugation to remove unbound LA. Then, LA capped AuNPs were re-suspended in ethanol and were stored at +4 °C for the further use [54]. The success of the reaction was confirmed measuring the size of AuNPs after coupling with LA.

2.3. Preparation of the nanoemulsions

All emulsions were prepared using the different percentages of the aqueous and the oil phase. For eight different formulations, the contents of both the aqueous phase and the oil phase were given in Table 1. To prepare the nanoemulsions, the aqueous and the oil phase were stirred using an ultrasonic processor (Vibra cell, Sonics & Materials, Inc., Newtown, USA) for 5 min with amplitude of 60%. All sonication processes were performed in an ice bath to avoid any thermal damage. The nanoemulsions were stored in the dark at +4 °C.