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Influence of basil oil extract on the antioxidant and antifungal activities of nanostructured carriers loaded with nystatin

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ABSTRACT

The combination of basil oil, natural antifungal, and nystatin has the potential to prevent the extension of topical fungal infections towards systemic infections. The aim of this study was to develop formulations based on basil oil and nystatin with the desired antifungal and antioxidant activity and low toxicity by using lipid nanocarriers. The synthesized nanocarriers showed spherical and homogeneous particles with main diameters less than 150 nm, as determined by TEM. The scanning calorimetric study revealed an imperfect crystallization in the core of lipid nanocarriers. Quantitative results suggested that basil oil concentration affects encapsulation efficiency. The prepared nanocarriers guaranteed an increased nystatin encapsulation by using 3% basil oil content. Chemiluminescence assay proved that the protective activity against oxygen free radicals was influenced by nystatin concentration. The in vitro antifungal studies revealed a better activity of the nanocarriers loaded with 1% nystatin in comparison with 0.5% loading.

1. Introduction

In recent years, despite the progress made in modern medicine, an increase in fungal infections was noticed [1,2]. The factors behind this increase include a weakened immune system, the treatment with corticosteroids and broad-spectrum antibiotics [3–5], the large number of cancers that imply a long-term chemotherapy [6], and the increased number of patients with chronic viral infections [7] and with various metabolic conditions [8,9]. The need to combat fungal infections makes the study of antifungal actives of great interest. Antifungals may be used in topical application for systemic mycoses. A fungal infection in immunosuppressed patients involves a high consumption of resources to eradicate fungi and a high risk of fatal complications [10]. Thus, a major goal is to prevent the progression of localized fungal infections towards systemic infections. This can be achieved by developing antifungal drugs with local action and minimal toxicity to the body, in order to limit and to eliminate the infections at the entrance gate [11,12]. Among antifungal drugs, the most important classes of antifungal compounds are polyenes, azole and echinocandins derivatives [13]. Many natural products, such as green tea [14], thyme [15,16], basil oil or other herbs proved to be effective in the treatment of topical fungal infections [17,18].

In this respect, two antifungals were selected in the present research to develop nanostructured lipid carriers (NLCs) able to combat fungal infections and free oxygen radicals, e.g., basil oil extract and a biosynthesized antibiotic, nystatin, produced by *Streptomyces noursei*. Nystatin belongs to the class of polyenes (Fig. 1), which

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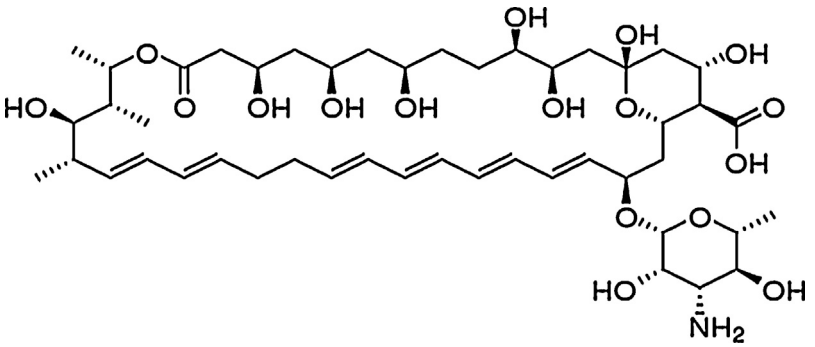

Fig. 1. Nystatin.

have been used in antifungal therapy for over 50 years, but their use is limited because of their high toxicity versus the human body [19,20]. Nystatin acts by irreversible binding to ergosterol and other specific sterols from the fungal cell membrane, leading to the formation of membrane pores through which ions are lost [13]. This leads to the destruction of the fungal cells.

Beside the antifungal action, the vegetable sources of active compounds could act as valuable natural antioxidants, and not only. The entrapment of vegetable extracts into various systems (e.g., liposome, nanostructured silica, lipid nanoparticles, etc.) has led to high fluorescent and improved antioxidant properties [21–25]. Nanostructured lipid carriers (NLC) are colloidal carrier systems composed of physiological lipid materials and surfactants accepted as GRAS [26,27] with high drug loading, encapsulation efficiency and stability [28–30] and, most importantly, they may increase bioavailability and stability of bioactive compounds and provide controlled release of encapsulated materials [31,32]. They have been accepted by regulatory authorities for application in cutaneous drug delivery (e.g., topical, dermal and transdermal) for both cosmetic and pharmaceutical areas [33,34].

The aim of this research was to synthetize lipid nanocarriers based on basil extract and loaded with Nys in order to reduce nystatin's toxicity and to increase their antifungal and antioxidant efficiency. Basil oil extract was used in this research with a dual purpose, firstly as natural antifungal and antioxidant agent, and secondly, as a component of the lipid matrix that will incorporate the antibiotic.

2. Experimental

2.1. Materials

Nystatin (Nys) was purchased from Antibiotics Iasi, Romania. Polyoxyethylene-sorbitan monooleate (Tween 80) was supplied by Merck (Germany). Synperonic PE/F68 (block copolymer of polyethylene and polypropylene glycol) and L-α-phosphatidylcholine were obtained from Sigma–Aldrich Chemie GmbH (Munich, Germany). Basil oil extract (BO) was purchased from Hofigal, Romania, and its fatty acid content has been determined by gas chromatography, using the derivatization method, which involves the transesterification of the oil with NaOH 0.5 N in methanol. Fatty acid methyl esters that were obtained after transesterification were subjected to gas-chromatographic analysis (Agilent Technologies, 7890A) coupled with mass spectrometry (Agilent Technologies, model 5975C VL MSD). The separation was performed on Supelco SP™ 2560 column (100 m, 0.25 mm internal diameter and 0.2 μm film thickness) with helium as the carrier gas (flow rate of 1.5 mL/min). The identification of the peaks was made by comparing the retention times with those of a standard mixture of 37 fatty acid methyl esters (FAME Mix Component Supelco™ 37). The fatty acids of BO were: 61.88% linoleic acid, 28.73% oleic acid, 5.60% palmitic acid, 3.45% stearic acid, 0.33% behenic acid. The solid lipids used were: *n*-hexadecyl palmitate (CP) purchased from Acros Organics (USA) and glyceryl monostearate (GMS) from Cognis GmbH.

2.2. Preparation of lipid nanoparticles

NLC were prepared using the melt emulsification coupled with high-pressure homogenization (HPH) method, according to the procedure described elsewhere [35]. Briefly, the aqueous phase containing a 2.5% surfactant mixture was heated under stirring at 85 °C. The lipid phase consisting of CP, GMS and BO was heated under stirring at 85 °C. The active ingredient, Nys, was added to the lipid phase and held for 15 min under stirring at the same temperature. Then, the lipid mixture was added to the aqueous phase, forming a pre-emulsion. The pre-emulsion was maintained under continuous stirring for 30 min at 85 °C, and then it was subjected for 2 min to high shear homogenization (HSH) at 10,000 rpm (Lab Homogenizer PRO250, 0–28,000 rpm, power of 300 W, Germany) and 5 min at 600 bar in a high-pressure homogenizer (HPH) (APV 2000 Lab Homogenizer, Germany). The formed emulsion was allowed to cool down at room temperature under continuous stirring to form a dispersion of the NLC loaded with Nys. The dispersion was freeze-dried in order to remove water by freezing for 24 h at –25 °C and then freeze drying for 3 days at –50 °C by using an Alpha 1-2 lyophilizer LD Freeze Drying System (Germany). The composition of the lipid carriers in the dispersion is shown in Table 1.