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Anti biofilm effect of dihydromyricetin-loaded nanocapsules on urinary catheter infected by *Pseudomonas aeruginosa*

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ABSTRACT

Nosocomial infections associated with biofilm formation on urinary catheters are among the leading causes of complications due to biofilm characteristics and high antimicrobial resistance. An interesting alternative are natural products, such as Dihydromyricetin (DMY), a flavonoid which presents several pharmacological properties, including strong antimicrobial activity against various microorganisms. However, DMY, has low aqueous solubility and consequently low bioavailability. Nanoencapsulation can contribute to the improvement of characteristics of some drugs, by increasing the apparent solubility and sustained release has been reported among other advantages. The aim of this study was to evaluate, for the first time, the feasibility of DMY nanoencapsulation, and to look at its influence on nanoencapsulation of DMY as well as verify its influence on antimicrobial and antibiofilm activity on urinary catheters infected by *Pseudomonas aeruginosa*. The physicochemical characterization showed an average diameter less than 170 nm, low polydispersity index, positive zeta potential (between +11 and +14 mV), slightly acidic pH. The values of the stability study results showed that the best condition for suspension storage without losing physical and chemical characteristics was under refrigeration (4 ± 2 °C). The antibiofilm activity of the formulations resulted in the eradication of biofilms both in free DMY formulations and in nanocapsules of DMY during those periods. However, within 96 h the results of the inhibition of biofilm by DMY nanocapsules were more effective compared with free DMY. Thus, the nanocapsule formulation containing DMY can potentially be used as an innovative approach to urinary catheter biofilm treatment or prevention.

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1. Introduction

Urinary tract infections associated with catheters are the most commonly acquired infections in the hospital environment [1]. These infections result in prolonged hospitalizations and high mortality rates [2].

The surface of the catheter acts as a platform for bacterial proliferation and biofilm formation because it provides ideal conditions for the development of biofilm populations. The deposition of urine into the catheter forms a protein film which increases the adhesion of microorganisms and facilitates the formation of biofilms [3–5]. Biofilm associated with the urinary catheter may become a potential risk for systemic infection and major complications such as pyelonephritis and sepsis [5–8].

Among the most frequently encountered pathogens when using a urinary catheter is *Pseudomonas aeruginosa*. These infections are usually hospital-acquired and may act as a reservoir in most hospital equipment and materials, especially with liquid components, which, combined with their natural resistance, can facilitate their distribution in the environment [9–12].

P. aeruginosa is associated with biofilm formation, which has a worse and is difficult to treat due to its intrinsic resistance to several drugs and therefore it is an important pathogen [13]. The high tolerance of *P. aeruginosa* biofilms to antimicrobial agents has been attributed to a combination of factors that contribute to the protection of the bacterial cells by exopolysaccharide matrix present in the biofilm [14]. The appearance of multiresistance strains is a matter of concern, and new therapeutic approaches must be found [15].

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It is extremely important to search for new therapeutic options, and natural products with antimicrobial activity have received increased attention due to their wide use, potential therapeutic effect and low incidence of adverse effects [5,16,17].

Dihydromyricetin (DMY), a flavonoid compound, is mainly extracted from *Ampelopsis grossedentata*, a wild plant from South China. It has low toxicity and different pharmacological properties, such as antioxidant, anti-tumor, lowering blood pressure, and is hypoglycemic, anti-thrombotic, anti-inflammatory and immunostimulatory [18–21]. Furthermore, DMY showed potential antimicrobial activity with significant inhibitory effects on *Staphylococcus aureus, Bacillus substillis, Aspergillus flavus, Penicillium* sp. and *Vibrio parahaemolyticus* [22,23].

However, the disadvantage of DMY is its low aqueous solubility, which contributes to poor tissue penetration and, consequently, low bioavailability [24]. Nanoencapsulation is an attempt at overcoming the obstacles of DMY.

Nanotechnology is one of the most important areas with the potential to solve problems caused by microbial infections [25]. Drug delivery by polymeric nanoparticles is considered a promising strategy to overcome the resistance of biofilms of *P. aeruginosa* [14,26]. Several studies have used nanotechnology to try to potentiate the antimicrobial activity of conventional drugs [1,13,26–32]. Nanoparticles can direct antimicrobial agents to the site of infection so that lower doses of the drug can be administered at the site of infection overcoming antimicrobial resistance without exceeding the systemic toxicity of the drug and preventing possible side effects [5,7,26,31–34].

The delivery of active compounds of polymeric nanocapsules is considered a promising strategy to overcome biofilm resistance. These nanocapsules are constituted by polymers which due to their structural characteristics allow a sustained release of the encapsulated drug maintaining plasma concentrations at therapeutic levels during certain periods of time, and are very important to control drug release [35–37]. These particles can improve antimicrobial delivery in the bacterial cell, increasing treatment efficacy [26,28,29,31,32].

Reports in the literature demonstrate the biosynthesis of gold nanoparticles using DMY, which resulted in gold nanoparticles with special shapes and different sizes that led to an important role in reduction during the synthesis process [67]. The result of this biosynthesis demonstrated significant antioxidant and antibacterial properties (for *Escherichia coli* and *Staphylococcus aureus*) in vitro [68].

However it should be emphasized that ours is the first work that allows the insertion of DMY in polymeric nanocapsules. Thus, this study aims to verify for the first time the feasibility of encapsulating DMY in nanocapsules and evaluating the influence of nanoencapsulation in antimicrobial and antibiofilm activities on *P. aeruginosa* infected urinary catheters.

2. Materials and methods

2.1. Materials

DMY (98% w/w) and acetonitrile grade-High performance liquid chromatography (HPLC) were obtained from Sigma-Aldrich (St. Louis, USA), ethanol grade-HPLC was acquired from Panreac (Barcelon, Spain). Eudragit RS100® was kindly donated by Evonik (Germany), A medium chain triglycerides mixture was obtained from Alpha Química (São Paulo, Brazil), Polysorbate 80 (Tween 80®) and acetone were provided by Synth (Diadema, Brazil). All chemicals and solvents were analytical grade and were used as received.

2.2. Pre-formulation studies

Initially, formulations were prepared at different concentrations of DMY. In order to choose the concentration of study for this DMY nanocapsules were prepared at concentrations 1.0 mg mL^{-1} (NC-1), 2.0 mg mL^{-1} (NC-2) and 5.0 mg mL^{-1} (NC-5). The formulations were evaluated for their physicochemical characteristics after 60 days of preparation in terms of particle size, polydispersity index, pH, value zeta potential and drug content. The samples were stored at room temperature (25° C ± 2° C) in amber glass flasks.

After choosing the best formulation considering stability, cost, and performance in biological pilot tests, nanocapsule suspensions were prepared at a final concentration of 1 mg mL^{-1} of DMY for further studies on characterization, stability and biological activity.

2.3. Preparation of nanoparticle suspensions

Nanocapsule suspensions were prepared by interfacial deposition of a preformed polymer, according to the method described by Fessi [38]. Eudragit RS100® (0.25 g) was solubilized in acetone (68 mL), DMY (0.025 or 0.050 or 0.125 g) and medium chain triglycerides (0.825 μL). After 20 min under moderate magnetic stirring at room temperature this organic phase was added to an aqueous phase (132 mL) containing polysorbate 80 (0.19 g). Magnetic stirring was maintained for 10 min at room temperature and then the organic solvent was eliminated by evaporation under reduced pressure to achieve a final volume of 25 mL. Blank nanocapsule suspensions (NC-B) were similarly prepared, omitting the presence of DMY.

2.4. Physicochemical characterization of nanocapsule suspensions and evaluation of stability

2.4.1. Determination of pH

The pH values of the suspensions were determined directly from the formulations using a previously calibrated potentiometer (Digimed® DM – 20) at room temperature. The results were expressed based on three different readings of the suspensions.

2.4.2. Particle size and zeta potential analysis

The mean particle sizes and polydispersity index (size distribution) were measured (n = 3) by photon correlation spectroscopy after dilution of an aliquot of nanoparticle suspension in ultrapurified water (1:500 v/v) employing a Zetasizer instrument (Zetasizer® Nano-ZS model ZEN 3600, Malvern Instruments, UK).

Zeta potential analyses were measured (n = 3) by electrophoretic mobility then performed in the same equipment after diluting the samples in a 10 mM NaCl solution (1:500 v/v).

2.4.3. Determination of drug content

DMY content was determined (n = 3) by High Performance Liquid Chromatography (HPLC) using a previously validated method. The nanocapsule suspensions that were used for the evaluation of all parameters were prepared at a concentration of 1 mg mL^{-1}. Nanocapsule suspensions (1.0 mL) were diluted with acetonitrile to a concentration of 100.0 μg mL^{-1} and subjected to ultrasonication (Unique®, Brazil) for 30 min. Subsequently, a portion of this solution (2.0 mL) was diluted with mobile-phase and again ultrasonicated for 30 min to yield a final concentration of 20.0 μg mL^{-1}. The resulting solution was filtered through a 0.45-μm membrane (Millipore®, Brazil) and injected into the HPLC system (n = 3). Chromatographic instruments and conditions were the following: Shimadzu HPLC system (Kyoto, Japan) was used and equipped with an LC-20AT pump, an SPD-M20A photodiode array (PDA) detector, a CBM-20A system controller, a C18 Phenomenex (4 × 3.0 mm) precolumn and

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