PRODUCTION AND BIOCOMPATIBILITY EVALUATION OF GERANYL CINNAMATE NANOEMULSIONS

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ABSTRACT

Geranyl cinnamate (GC), an FDA-approved compound known for its antimicrobial and antioxidant properties, faces limitations in its application due to low solubility in aqueous media. In this context, the objective was to develop a polymeric nanoemulsion (NE) with GC as the oil-core and assess its *in vitro* biocompatibility. Dynamic Light Scattering yielded promising results for the NE, describing a monodisperse system with submicron particle size. The Zeta Potential measured (-34.4 ± 1.0 mV) indicates a stable nanocarrier system. Geranyl cinnamate ester in its free and nanoemulsified form exhibited no apparent cytotoxicity against the fibroblast lineage (L929) within the tested concentration range. Overall, these findings suggest a promising NE suitable for application in the cosmetic and pharmaceutical industries.

Keywords: Bioactive compound. Nanocarrier. Cinnamic Acid. Geraniol. Cytotoxicity.

1 INTRODUCTION

Esters derived from monoterpene alcohols have been investigated for generating biomolecules that offer advantages such as greater molecular stability and lower volatility compared to their precursor alcohols[1]. Among these derivative esters is Geranyl cinnamate (GC).

Geranyl cinnamate, an FDA-approved compound, is utilized as a flavoring agent in food products[2]. Additionally, it possesses antimicrobial and antioxidant properties, providing further advantages[3]. However, the application of GC is still limited due to its low solubility in aqueous media[2].

In this context, one possible solution is to utilize nanostructuring methods such as nanoemulsification. The application of biomolecules carried by nanostructured systems improves the compound's solubility, potentially bringing features like enhanced availability of the molecule in biological systems and controlled release, characteristics desired by the pharmaceutical and cosmetic industries[4].

For the use of nanoemulsified actives in the industry, it is important, however, to assess the cytotoxicity of the nanoemulsified compound[5]. This is because if used in the formulation of any product, it should not endanger the health of consumers[6]. Therefore, evaluating the cytotoxicity of compounds and derivatives is an important test for their utilization.

Thus, the aim of the study was to develop a polymeric nanoemulsion (NE) using geranyl cinnamate ester as the oil-core, as well as to evaluate the in vitro cytotoxicity of the compound in its nanostructured form.

2 MATERIAL & METHODS

Geranyl cinnamate ester was previously synthesized and purified according to Remonatto et al. (2022)[1] modified. For the synthesis, geraniol 97%, Sigma-Aldrich, St. Louis, MO, USA), cinnamic acid 99%, Sigma-Aldrich, St. Louis, MO, USA) and the lipase (Novozyme® 435) were used. The synthesis occurred inside a jacketed glass batch stirred tank reactor (10 cm height and 4.5 cm diameter) connected to a thermostatic bath (Marconi, model MA 184/6, Piracicaba, SP, Brazil). The conditions were 1:5 (geraniol to cinnamic acid) substrate molar ratio, 65 ºC and 15 % (w/w) of enzyme loading.

To obtain a reservoir-type nanocarrier system with a lipophilic core (containing geranyl acetate ester as the oil-core), the nanoprecipitation method was applied according to De Paula et al. (2017) modified[7]. The NEs were synthesized at a concentration of 1.25% (w/v) of geranyl acetate.

For physicochemical characterization, the particle size, polydispersity index (PdI), and zeta potential (ζ) were determined using Dynamic Light Scattering (DLS) technique. The analyses were conducted 1, 7, and 27 days after synthesis.

The evaluation of the biocompatibility of geranyl cinnamate ester in its free form, its nanoemulsified form, and the oil-core-free nanoemulsion was conducted on fibroblast lineage (L929), utilizing the cellular viability marker resazurin according to Miotti et al. (2024)[5].

3 RESULTS & DISCUSSION

Based on the presented data (Table 1), the nanoemulsion developed with geranyl cinnamate (NE/GC) exhibited particle sizes greater than 100 nm, which are satisfactory values for nanoemulsions of polymeric materials. The results of these analyses showed that the polymeric formulations exhibit the desired physicochemical parameters, with particle size and zeta potential indicating stability during the analysis period.

Table 1 Mean particle size, polydispersity index (PdI), and zeta potential of the Geranyl Cinnamate nanoemulsion (NE/GC)

	Mean particle size (nm)	PdI	Zeta Potential
NE/GC	302.0 ± 119.9	0.6 ± 0.3	-34.4 ± 1.0

Although the PdI indicates that the system has some polydispersity, it is shown in Figure 1 that the NE/GC has its nanocarriers size concentrated in one region of the distribution which indicates a monodisperse system.

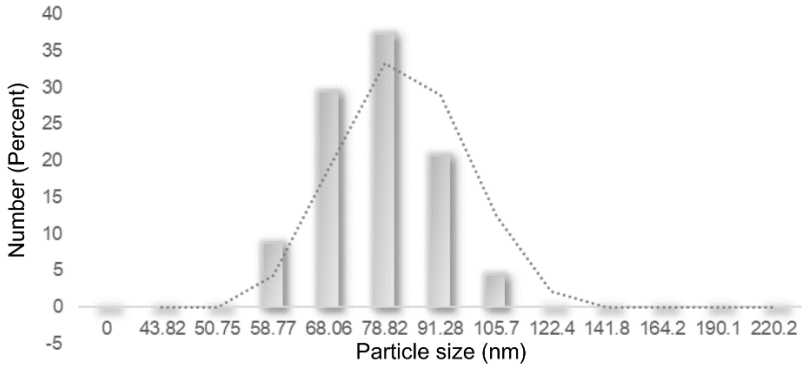

Figure 1 Particle size distribution of the nanoemulsion synthesized with Geranyl Cinnamate

As depicted in Figure 2, both the nanoemulsions produced with geranyl cinnamate (NE-GC) and the oil-core-free nanoemulsion (NE) showed good viability, indicating low cytotoxicity on the basal lineage (fibroblasts, L929) tested.

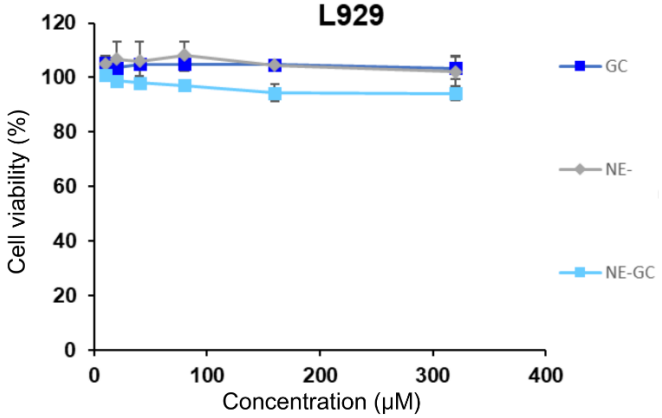

Figure 2 In vitro biocompatibility study of free and nanoemulsified geranyl cinnamate on basal fibroblast cell line (L929).

Nanoemulsifying the geranyl ester has proven to be a good solution to the current challenge regarding the molecule's low solubility. Thus, with the initial test on the biocompatibility of the nanoemulsion, it is evident that there is great potential for its application in the pharmaceutical and cosmetic industries. Future studies on the formulation stability of the nanoemulsion are necessary, as well as further tests on the biocompatibility of the ester with other cell lineages.

4 CONCLUSION

The method employed for the synthesis of the geranyl cinnamate nanoemulsion was satisfactory. A monodisperse nanocarrier system was obtained. Based on the studies of cellular biocompatibility, it was possible to conclude that the ester, especially in its nanoemulsified form, holds significant potential for application in pharmaceutical and cosmetic products.

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