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EVALUATION OF THE STABILITY AND ECOTOXICOLOGICAL PROFILE OF NANOSTRUCTURED LIPIDIC SYSTEMS CONTAINING GINGER ESSENTIAL OIL COMPARED TO ITS FREE FORM[1]

AVALIAÇÃO DA ESTABILIDADE E PERFIL ECOTÓXICO DE SISTEMAS LIPÍDICOS NANOESTRUTURADOS CONTENDO ÓLEO ESSENCIAL DE GENGIBRE COMPARADO COM SUA FORMA LIVRE

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ABSTRACT

The ginger essential oil presents medicinal properties of interest, but it has a low solubility and a high toxicity, which limits its use in the health area. Through the production of nanostructured systems, these damages can be minimized without reducing their medicinal properties. The bioassays conducted on ecotoxicity with Artemias salina, the free ginger essential oil, and nanostructured systems of the lipidic carrier type aimed to check if these systems can protect the active, in order to improve its bioavailability and to reduce its toxicity. The evaluation of ecotoxicity with Artemias salina showed a significant reduction in the toxicity of the nanostructured systems when compared to the positive control and the oil in its free form. Given the results obtained in this study, we can conclude that the nanostructured systems tested do not present ecotoxicity and therefore have great potential for use in nanomedicine, thus contributing to the use of products of plant origin in nanotechnology when applied to health care.

Keywords: bioassays, ecotoxicity, nanotechnology.

RESUMO

O óleo essencial de gengibre apresenta propriedades medicinais de interesse, porém possui baixa solubilidade e alta toxicidade, o que limita sua utilização na área da saúde. Através da produção de sistemas nanoestruturados pode-se minimizar esses danos sem reduzir suas propriedades medicinais. Neste estudo foram realizados testes de ecotoxicidade através de bioensaio com Artemia salina, utilizando o óleo essencial de gengibre livre, e sistemas nanoestruturados do tipo carreadores lipídicos, com o objetivo de verificar a capacidade destes sistemas nanoestruturados em proteger o ativo, melhorando a sua biodisponibilidade e reduzindo sua toxicidade. A avaliação de ecotoxicidade com Artemias salina mostrou significativa redução da toxicidade dos sistemas nanoestruturados quando comparados ao controle positivo e ao óleo na sua forma livre. Diante dos resultados obtidos neste estudo, podemos concluir que os sistemas nanoestruturados testados não apresentam ecotoxicidade e têm, portanto, grande potencial para utilização em nanomedicina, contribuindo assim com a utilização de produtos de origem vegetal na nanotecnologia aplicada a saúde.

Palavras-chave: *bioensaios, ecotoxicidade, nanotecnologia.*

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INTRODUCTION

Several types of essential oil are widely used because they have major constituents, which can be easily isolated and identified (ROMOFF et al., 2010). Ginger (Zingiber officinale Roscoe) is a plant originally from Southeast Asia, which has been used in Chinese and Indian millenarian cuisines and is appreciated in the modern world. The rhizome is marketed fresh, dried, chopped, preserved, crystallized, powdered or as an essential oil and it has a spicy and slightly bittersweet taste (PANIZZA, 2001; ELPO; NEGRELLE, 2006).

It is popularly known for its medicinal use (ELPO; NEGRELLE, 2004; MENDES, 2005). In addition to the sensory properties, aromatic plants, such as ginger, are used as preservatives, antioxidants and antimicrobiotics (SINGH et al., 2008). They are invaluable sources of new drugs, and their essential oil and constituents have been widely used by the pharmaceutical industry, among other functions, in viral and bacterial combat, as well as in the fight against cancer and diabetes (NOGUEIRA DE MELO et al., 2011). Although ginger stands out for its application in the food industry as a natural preservative (BEAL, 2008), it is also used in diet due to its properties as anti-inflammatory, antiemetic, anti-nausea, antimutagenic, food detoxifying, antiulcer, hypoglycemic, bactericidal and as a general tonic (YOSHIKAWA; YAMAGUCHI; KUMINI, 1994; AMEENAH, 2002; BULLETIN, 2003; SACCHETTI, 2004; GEIGER, 2005; FERNANDES, 2006; ZHOU et al., 2011; SUKLA, 2007). Because of its high anti-inflammatory activity, it has a great potential for modulating leukocyte recruitment (NOGUEIRA DE MELO et al., 2011). Ginger has a great potential against the pain of women's primary dysmenorrhea. Its oil stands out in its ability to inhibit cell damage (PEREZ-ROSES et al., 2016). *Zingiber officinale* has benefits in the treatment of musculo-skeletal disorders, vomiting, inflammation, osteoarthritis, migraine, cancer, hyperlipidemia, and hyperglycaemia (MORAKINYO; AKINDELE; AHMNED, 2011). Some of the various constituents of ginger have an essential effect on the potency of tumor growth through gene organization, induction of cellular self-destruction, apoptosis, or impediment of growth of new blood vessels (AL-TAMIMI; RASTALL; ABU-REIDAH, 2016). In vitro studies with the ginger root rhizome powder and one of its main components, gingerol, report the inhibition of the synthesis of several proinflammatory cytokines (RAMADAN; AL-KAHTANI; ELSAYED, 2011). The uses of essential oils, extracts and concentrates of *Zingiber officinale* are described in traditional medicine and have aroused the interest of the pharmaceutical industry in developing laboratory analyzes in order to identify and scientifically verify the active principles and their functions in the human organism (MORAKINYO; AKINDELE; AHMNED, 2011).

The main limiting factor for its use in medicine is the fact that essential oils, such as lipophilic compounds, present a high potential for toxic interference (SIMPSON; OGORZALY, 1995). They are complex mixtures synthesized by plants. In general, essential oils are unstable, very sensitive to the

presence of light, heat, moisture, air, oxidizing, reducing substances and metals (BAKKALI, 2008). According to Antunes et al. (2017), the encapsulation, production of nanofibers or other biosystems are alternatives to reduce the limitations of the use of essential oils such as the ginger one, which is a necessity, given the difficulties of its use in the traditional way, as it improves solubility, stability and enables the controlled release of compounds (RESTUCCIA et al., 2010).

Nanotechnology is a set of techniques of manipulation of matter, which has been advancing rapidly in the scientific environment, opening up numerous possibilities in biotechnology and in virtually all areas of knowledge. Nanostructured systems are a promising alternative for biomedical applications. Through nanotechnology, some limiting characteristics of certain assets such as instability, low solubility in water and photosensitivity can be bypassed, in addition it can protect the active and reduce its toxicity. The use of nanostructured systems is also related to a higher concentration in the sites of action, thus reducing the adverse reactions related to high doses used in the systemic route (TORCHILIN, 2007). Encapsulation and delivery systems of bioactive compounds, due to their biodegradability and biocompatibility, are in great demand today (FATHI; MARTÍN; MCCLEMENTS, 2014).

The lipid-based nanoparticles are a type of carrier composed of a matrix whose lipid is solid at ambient temperatures and contain lipids and surfactants with high biocompatibility (FREITAS; MÜLLER, 1999; SEVERINO et al., 2012). Nanostructured lipid carriers are considered to be an enhancement of solid lipid nanoparticles, since, during storage, the active molecules incorporated in the solid lipid matrix are expelled (INOVA, 2017). After the emergence of solid nanolipids, a new generation of nanostructured lipid carriers has been developed, known as lipid nanocarrier systems (LNS), which are being considered a new type of lipid nanoparticle formed by a mixture of solid and liquid lipids. This nano system presents imperfections in its crystalline solid structure, caused by the presence of the oil (liquid lipid) (MÜLLER et al., 1995). The production of nanostructured systems can increase the effectiveness of these systems, since in the nanostructured form they have a greater surface area, which promotes their greater reactivity in significantly reduced doses, making studies of alternative therapies potentially usable in biomedicine. The main advantages of lipid carriers over other traditional drug carriers are the greater biocompatibility, lower cytotoxicity, good scalability of production, modulation of drug release, without the use of organic solvents in the preparation process, and a broad spectrum of application routes (dermal, intravenous, etc.) (MONTENEGRO et al., 2016). According to studies by Carvalho et al. (2009), Artemia salina is used in acute toxicity tests due to its ability to form dormant cysts, its practical handling and cultivation, for being a fast and cheap method, applicable as a bioindicator in a toxicological evaluation. The bioassay with Artemia salina has been used in different analyzes of toxicity of substances, and like other techniques of analysis, it is also efficient in the analysis of nanostructured systems. Polymeric systems for drug delivery are widely used as they allow for a slow and gradual release of the active substance, and potentially aiming at

specific targets in the body to be treated. Nanoscale studies with ginger oil as an active ingredient are still restricted, which makes the present work a significant contribution to the scientific environment.

MATERIAL AND METHODS

PRODUCTION AND STABILITY EVALUATION OF NANOSTRUCTURED SYSTEMS

For the production of lipid nanocarrier systems, it was used the method described by Raffin et al. (2012), in which the nanoparticle suspensions were prepared using a high shear mixer. The lipid phase was composed of shea butter, sorbitan monooleate, and ginger essential oil while the aqueous phase was composed of polysorbate 80 and ultrapure water. The lipid phase and aqueous phase were simultaneously heated at 40 °C under constant magnetic stirring until all components were melted. Thereafter, the aqueous phase was poured onto the lipid phase under stirring. The suspension was subjected to stirring in the high shear mixer at 17.000 rpm for 20 minutes. The stability of the systems was evaluated through the analysis of the following physicochemical parameters: hydrogen potential (pH), zeta potential, Polydispersion index (PDI) and average particle diameter. The results are shown in table 1.

Table 1 - Composition of blank nanostructured lipid (NLB), and nanostructured lipid containing ginger essential oil (NLG).

Constituents	NLB	NLG
	Oil Phase	
Crodamol Oil ™ (g)	1	
Ginger oil		1
Shea butter (g)	4	4
Span 80	2	2
	Water Phase	
Tween 80 (g)	3	3
H_2O mQ (mL)	89	89

BIOASSAY WITH *ARTEMIA SALINA*

In order to perform the bioassay using *Artemia salina*, it was used the method of hatching the cysts and obtaining nauplii from Artemia sp. for the toxic response experiment, according to the procedure described by Veiga et al. (1989). 10 mg *Artemia salina* eggs were used with 90% hatching, 8000 mL becker, an aquarium pump to aerate the system and a 18 watt white light bulb. Artificial marine water - prepared according to Dietrich e Kalle's formula: for 1 L of distilled water, add 23 g of NaCl, 11 g of $MgCl_2 \times 6H_2O$, 4 g of Na_2SO_4, 1.3 g of $CaCl_2 \times 2H_2O$ and 0.7 KCl; adjust the pH of the solution to 9.0 with Na_2CO_3. For the hatching of the cysts, a 800 mL becker containing 500 mL of artificial marine water was used, protected with foil leaving only a small opening on the upper surface, illuminated by an 18 W lamp placed at a distance of approximately 30 cm. The aeration was promoted

to water for 15 minutes before placing the cysts and aeration was kept during the whole incubation time at 28 ºC, salinity of 32 µg/mL and pH 9.0. The agglutination of the cysts alters the hatching so it is important to keep them in constant movement with aeration from the base of the becker. For the determination of acute toxicity and LC50, solutions with concentrations 5.0; 10; 15 and 20 mg/L were prepared, diluted in artificial marine water, and it was used a negative control consisting of artificial marine water without the test substance and a positive control consisting of DMSO diluted in artificial marine water at the highest tested concentration (20 mg/L). The bioassay was organized in test tubes containing 10 mL of test or control solution, to which 10 nauplii were added and kept for 48 h at 25 °C. After 24h and 48h, the number of live nauplii was counted, and any abnormal behavior was noted (e.g. difficulties in swimming). Data were analyzed statistically by the BIOSTAT software, and the results were expressed by mean and standard deviation. All tests were done in triplicate.

METHODOLOGY TO CALCULATE THE LC50 OF THE SAMPLES TESTED

The LC50 was calculated using PROBITS and LOG. From the concentration applying the simple linear regression statistic, where the data obtained for intercept (a) and regression coefficient (b) the formula used was : $Y1 = a = bX$.

STATISTICAL ANALYSIS

The statistical program GraphPad Prisma version 5.0 was used to perform the statistical analyzes and the data were expressed as mean ± standard deviation (SD). All in vitro experiments were conducted in triplicate and treatments were compared through one-way analysis of variance followed by Dunnett's post hoc test. Tests with $p < 0.05$ were considered significant. Being that * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. For LC50 calculations, the data collected were statistically analyzed by the BIOSTAT software, and the results were expressed by mean and standard deviation. All tests were done in triplicate.

RESULTS

RESULTS OF STABILITY ANALYSIS OF SUSPENSIONS

The results of stability analysis of suspensions containing blank nanostructured lipid carriers and lipid carriers containing ginger essential oil are being presented in the tables 2 and 3.

Table 2 - Stability of blank nanostructured lipid (NLB) on physicochemical parameters.

	DAYS	pH	Mean diameter (nm)	PDI	Zeta Potential (mv)
NLB	0	5.2 ± 0.0	94 ± 0.0	0.20 ± 0.0	-14 ± 0.2
	3	5.2 ± 0.2	94 ± 0.0	0.18 ± 0.1	-16 ± 0.0
	7	5.1 ± 0.2	95 ± 0.1	0.19 ± 0.1	-16 ± 0.0
	20	5.1 ± 0.2	94.8 ± 0.1	0.20 ± 0.0	-16 ± 0.0
	30	4.9 ± 0.2	94.3 ± 0.1	0.20 ± 0.0	-16 ± 0.0
	40	4.9 ± 0.0	95 ± 0.1	0.20 ± 0.0	-16 ± 0.0
	60	4.9 ± 0.0	95 ± 0.1	0.20 ± 0.0	-16 ± 0.0
	90	4.9 ± 0.0	98 ± 0.1	0.20 ± 0.0	-16 ± 0.0

Data represent means ± standard deviation (n = 3, for each day). They were analyzed by one-way Anova, followed by the Tukey test. * P <0.05 when compared to day 0 (zero). There were no statistically significant differences for these data. Where: blank nanostructured lipid (NLB); pH - Hydrogen potential, PDI - index of polydispersity, and Zeta Potential (mv). Source: Prepared by the author.

Table 3 - Stability of ginger nanostructured lipid (NLG) on physicochemical parameters.

	DAYS	pH	Mean diameter (nm)	PDI	Zeta Potential (mv)
NLG 1mg/mL	0	4.8 ± 0.2	91.7 ± 0.1	0.246 ± 0.1	-10.3 ± 0.0
	3	4.9 ± 0.2	91.7 ± 0.1	0.24 ± 0.0	-10.3 ± 0.0
	7	4.9 ± 0.2	74.4 ± 0.2	0.204 ± 0.0	-10.1 ± 0.0
	20	4.5 ± 0.2	74.0 ± 0.1	0.24 ± 0.0	-10 ± 0.0
	30	5.1 ± 0.1	74.0 ± 0.08	0.24 ± 0.1	-10 ± 0.0
	40	5.1 ± 0.1	74.0 ± 0.1	0.20 ± 0.0	-10 ± 0.0
	60	5.3 ± 0.1	78.0 ± 0.1	0.20 ± 0.1	-10 ± 0.0
	90	5.3 ± 0.1	78.9 ± 0.1	0.20 ± 0.1	-10 ± 0.0

Data represent means ± standard deviation (n = 3, for each day). They were analyzed by one-way Anova, followed by the Tukey test. * p <0.05 when compared to day 0 (zero). There were no statistically significant differences for these data. Where: ginger nanostructured lipid (NLG); pH: hydrogen potential, PDI: polydispersity index, and and Zeta Potential (mv). Source: Prepared by the author.

RESULT OF CALCULATING LC50

The results of calculating LC50 of suspensions containing blank nanostructured lipid (NLB) and ginger oil nanostructured lipid are presented on the table 4.

Table 4 - CL 50 result of free ginger essential oil and nanostructured systems containing ginger essential oil.

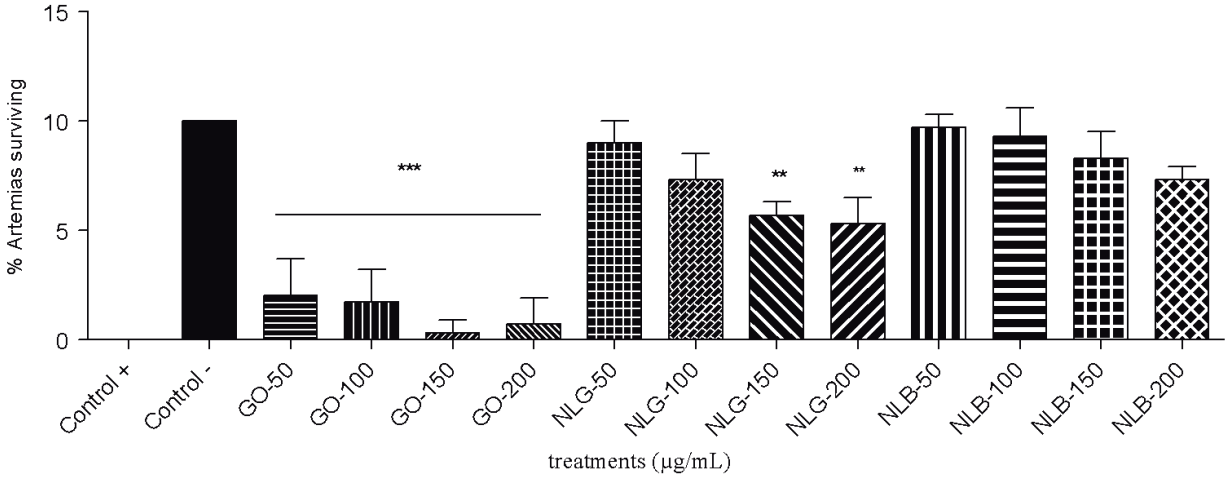
Test sample	LC50
GO	1.94µL/mL
NLB	6300 µL/mL
NLG	7.58 µL/mL

Where: NLG: ginger *oil* nanostructured lipid, NLB: blank nanostructured lipid and GO: ginger oil
Source: Prepared by the author.

RESULTS ACUTE ECOTOXICITY BIOASSAY WITH *ARTEMIA SALINA*

The results of the acute ecotoxicity bioassay with *Artemia salina* are shown on figure 1.

Figure 1 - Results obtained by the ecotoxicity test with *Artemia salina* with free ginger essential oil and ginger oil in nanostructured systems.



Acute toxicity bioassay with *Artemia salina*. Results expressed as percentage of negative control (100%). Data were expressed as mean ± standard deviation (SD). Analyzes were performed by one-way ANOVA, followed by Dunnett's post hoc test. Values with $p < 0.05$ were considered statistically significant (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$). Where C-: negative control, C +: positive control, GO: free ginger oil, NLG nicarreador lipid containing ginger oil and NLB: white lipid carrier.
Source: Prepared by the author.

DISCUSSION

Artemia salina bioassays have a great benefit for studying the toxicities of nanostructures, besides having a great availability of their eggs, they also present fast, simple and reliable results (SOMAYEH; RAMAZANI; TAHEREH, 2015). Our results demonstrate a high toxicity of the free oil on the mortality rate of the *Artemia*, clearly related to the increase in the concentration of the active, a result that corroborates with a previously reported study (KAZEEM et al., 2012), which reports that the higher the concentration of the active substance (ginger) the greater its toxicity. In contrast, the toxicity of the nanostructured systems was clearly reduced, evidencing a potential environmental protection. In our study, the production method used was efficient in the production of lipid nanocarriers with pH, PDI, diameter and zeta potential suitable for the usage in the health area. Nanocapsules and nanoemulsions containing essential oil of ginger have already been developed and presented physical-chemical stability for 60 days (PINTO et al., 2017). Our results demonstrate formulations with slightly acid pH, between 4.8 and 6.0. This result was already expected by the character of the constituents. The mean diameter of the particles was between 74 and 98 nanometers and the mean PDI was between 0.18 and 0.24, results that are in accordance to those found in previous

studies (PARDEIKE; HOMMOSS; MÜLLER, 2009; DATE et al., 2017). Our results indicate that the suspensions produced presented some stability characteristics in the analyzed period of 90 days.

These results point to a potential use of the formulations produced in this study in Nanomedicine, since they have some characteristics also evidenced in other nanoparticles of already consecrated use. The bioassay using *Artemias salina* is an efficient, fast and relatively low cost methodology (CETESB, 1991; SIQUEIRA et al., 1998; MOREIRA et al., 2003). In a study with ornamental plants, Machado (2003) observed that of the eleven plants tested, five presented significant toxicity value to *Artemias salina: Ruta graveolens, Sansevieria trifasciata*, *Zantedeschia aethiopica, Nerium ollander and Hedera helix*. Benassi (2004) used the bioassay with *Artemia* sp as a bioindicator and biomarker against the leaching of mineral coal. In the acute ecotoxicity test containing ginger essential oil, when compared to the toxicity of the active in the free form, which makes us believe in the ability of these nanostructures to protect the environment.

FINAL CONSIDERATIONS

Our results allow us to conclude that the nanostructured systems containing ginger oil had their toxicity reduced when compared to the essential oil of free ginger. This finding attests the potential of nanotechnology to develop nanostructured biosystems with assets of vegetal origin for use in Nanomedicine with environmental security.

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