INTRODUCTION

Arthritis is a painful and disabling condition that involves inflammation of one or more joints, with infiltration of inflammatory cells, synovial hyperplasia, cartilage destruction, bone erosion, narrowing of the joint space, and ankylosis of the joint (Bendele et al., 1999). The most common forms of arthritis are osteoarthritis, rheumatoid arthritis, and gout, the latter of which is responsible for the worst episodes of acute pain (Cannella, Mikuls, 2005). The pharmacological treatment of arthritis involves reducing joint pain and inflammation and/or slowing down the progression of the disease through administration of drugs such as analgesics, corticosteroids, disease-modifying antirheumatic drugs (DMARDs), non-steroidal anti-inflammatory drugs (NSAIDs), and biological drugs (Negrei et al., 2016). However, some of these drugs exhibit severe adverse effects that make patient compliance difficult. This shortcoming has stimulated research into new arthritis treatments, and a great variety of active compounds for developing new medicines can be found in plants (Ghosh et al., 2016; Khanna et al., 2007).

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treatment for arthritis (Basualdo, Zardini, Ortiz, 1991). In fact, in a rat model of gout induced by monosodium urate (MSU) crystals, a hydroalcoholic extract (70% v/v) obtained from the underground parts of *J. isabellei* demonstrated antinociceptive and anti-inflammatory properties, although this treatment was not able to alter the increased levels of uric acid produced in a hyperuricemia model using potassium oxonate (Silva *et al.*, 2013). The underground parts of *J. isabellei* have been chemically characterized as possessing terpenic compounds. In particular, diterpene jatrophone has been found to be an important constituent of the dichloromethane fraction (DF$_{J_i}$) obtained from hydroalcoholic extracts of *J. isabellei*, alongside minor amounts of acetyl aleuritolic acid and a binary mixture of sitosterol-3-O-β-D-glucoside and stigmasterol (Fröhlich *et al.*, 2013). The administration of DF$_{J_i}$ orally or intravenously, significantly reduced the rat paw elevation time (PET) and edema formation following intra-articular injections of carrageenan in an induced acute arthritis model in rats, evidencing this fraction possesses both antinociceptive and anti-inflammatory properties (Fröhlich *et al.*, 2017). In this same study, they found the pharmacological effectiveness of DF$_{J_i}$ to be higher following intravenous administration opposed to the oral route, indicating limited absorption of DF$_{J_i}$ chemical compounds via the gastrointestinal tract.

Thus, despite its beneficial pharmacological properties, DF$_{J_i}$ is composed of poorly water-soluble compounds that exhibit slow drug absorption and, thus, poor and erratic bioavailability. An interesting approach to overcoming this drawback is to consider lipid-based formulations for administering DF$_{J_i}$ by the oral route. The ability of lipids to enhance the bioavailability of lipophilic drugs has been ascribed to a lipid-sensing mechanism in the gastrointestinal tract, stimulation of which induces the secretion of gastric and pancreatic lipases and bile salts. Lipases digest the oil component of nanoemulsions into simpler lipids (diglycerides, monoglycerides, and fatty acids), which then form mixed micelles with bile components, thus solubilizing the free drug and carrying it across aqueous unstirred diffusion layer for absorption. Occasionally, nanoemulsion droplets may be absorbed intact, either via paracellular or transcellular pathways, or via M-cells present in Peyer’s patches or other absorptive cells. Once inside an absorptive cell, nanoemulsions can be processed into apolipoproteins, thus gaining access to the intestinal lymphatic system and effectively bypassing the liver (Singh *et al.*, 2017).

Colloidal lipid carriers such as nanoemulsions, solid lipid nanoparticles, and lipid nanocapsules, although being formed from different kinds of lipids and surfactants and exhibiting different structures, all present one common characteristic in their nanometric particle size. In addition to increasing the intrinsic solubility and bioavailability of lipophilic drugs, entrapping drug substances in lipid nanocarriers (LNCs) helps protect against their degradation by gastrointestinal fluids. Finally, lipid carriers have the advantage of being less toxic than polymeric nanoparticles, and generally include compounds holding Generally Recognized as Safe status for oral administration (Cai *et al.*, 2010; Pimentel-Moral *et al.*, 2018).

Given these considerations, this paper describes the preparation and characterization of LNCs containing DF$_{J_i}$ (LNC$_{J_i}$) designed to improve the oral absorption of the bioactive compounds. The *in vitro* jatrophone release and accelerated stability studies of the LNCs are described. Additionally, the *in vivo* pharmacological properties of LNC$_{J_i}$ are evaluated, alongside a free suspension of DF$_{J_i}$, via oral administration in a rat model of carrageenan-induced arthritis.

**MATERIAL AND METHODS**

**Material**

Medium chain triglyceride oil (Ritamollient CCT) was acquired from Brasquim (Brazil). Hydrogenated soybean lecithin (Lipoid S100, 97.5% phosphatidylcholine) and egg lecithin (Lipoid E80, 80% phosphatidylcholine) were purchased from Lipid Ingredients & Technology (Brazil). Polysorbate 80 (Tween 80) was acquired from Sigma-Aldrich (USA). The combination of kappa/lambda carrageenan was purchased from BDH Chemicals Ltd. (UK). Dexamethasone was purchased from Deg (Brazil). Water utilized in the HPLC analyses was obtained using a Milli-Q purification system (Millipore, USA). With the
Plant collection and preparation of dichloromethane fraction dry extract

*Jatropha isabellei* Müll. Arg. (family Euphobiaceae) was collected in the municipality of Cacequi (State of Rio Grande do Sul, Brazil, coordinates: 29°53’01” S and 54°49’30” W) and identified by Professor Renato Záchia in May of 2008. An exsiccate was archived in the herbarium of the Biology Department at the Federal University of Santa Maria (SMDB 11816). The underground parts of the plant were dried at room temperature and powdered in a knife mill. The powder was macerated with 70% (v/v) ethanol using a plant to solvent ratio of 1:3 (w/v) for ten days at room temperature. After filtration, the ethanol was evaporated under reduced pressure and the resulting suspension was partitioned with dichloromethane. The dichloromethane fraction was then further taken to dryness under reduced pressure, resulting in the dichloromethane fraction dry extract (DF$_{Ji}$, yield 3.7%).

Analysis of DF$_{Ji}$ fatty acids

The fatty acid composition of the DF$_{Ji}$ was determined using gas chromatography with flame ionization detection (GC-FID) following conversion of the lipid material into the corresponding methyl esters, according to the method described elsewhere (Christie, 1993). The sample was analyzed using an Agilent 7820A gas chromatography system equipped with a flame-ionization detector. A CP-Sil 88 fused silica capillary column (100 m X 0.25 mm ID X film thickness 0.20 µm) was used to separate the compounds. Injector and detector temperature were kept at 250 °C and the injection volume was 1.0 µL with a split ratio of 1:50. Hydrogen was used as the carrier gas with a constant flow of 1.0 mL/min and nitrogen was used as an auxiliary gas (30 mL/min). The oven temperature was set to 45 °C for 4 min, then increased to 175 °C at a rate of 13 °C/ min, held at 175 °C for 27 min, then increased by 4 °C/ min to 215 °C and held at that temperature for 35 min (Cruz-Hernandez et al., 2007). Peaks were identified by comparison of their retention times with those of a Supelco 37-component fatty acid methyl ester mix (FAME, Sigma-Aldrich, USA).

Preparation of lipid nanocarriers

LNCs containing the dichloromethane fraction dry extract from *J. isabellei* (LNC$_{Ji}$) were prepared using the spontaneous emulsification method (Bouchemal et al., 2004). Briefly, an organic phase containing 100 mg of medium chain triglyceride oil (MCT), 10 mg of soybean lecithin, and 100 mg of DF$_{Ji}$ in 10 mL of acetonitrile (7.3 v/v) was slowly added to 50 mL of an aqueous phase containing 0.5% polysorbate 80, under vigorous magnetic stirring. Next, the organic solvents were removed by evaporation under reduced pressure and the colloidal dispersions were concentrated to a final volume of 10 mL. Unloaded lipid nanocarries (LNC$_{blank}$) were prepared using identical conditions but without DF$_{Ji}$ added. All formulations were prepared in triplicate.

Characterization of lipid nanocarriers

Particle size and zeta potential

The mean particle diameter and zeta potential were determined by dynamic light scattering and laser-Doppler anemometry, respectively, using a Zetasizer Nano Series (Malvern Instruments, UK). The measurements were performed at 25 °C after appropriate dilution of the samples in ultrapure water (Milli-Q®, Millipore, USA). Each size analysis lasted 100 s and was performed with an angle detection of 173°. For measurement of zeta potential, the diluted samples were placed in the electrophoretic cell where a potential of ± 150 mV was established. The zeta potential values were calculated as mean of electrophoretic mobility values using Smoluchowski’s equation (Sze et al., 2003).

pH measurements

The pH of LNC dispersions were determined using a pH meter (Oakton pH 5 Acorn Series, USA), previously calibrated with buffer solutions of pH 4.0 and 7.0.
Transmission electron microscopy

After appropriate dilution of the samples, the morphology of the LNC$_{Ji}$ was examined using a JEM-1011 transmission electron microscope (JEOL, Tokyo, Japan) with an acceleration voltage of 100 kV. The nanoparticles suspensions were dropped onto carbon-coated copper grids and visualized by staining with 1% phosphotungstic acid.

Determination of jatrophone loading and encapsulation efficiency

The jatrophone loading into the LNCs was determined using a previously developed and validated high performance liquid chromatography (HPLC) method with UV-visible diode-array detection (Fröhlich et al., 2017). The analyses were performed using a Shimadzu UFLC system (Japan) equipped with a LC-20AD binary pump, a SIL-20AC HT auto-sampler, a CTO-20A forced air-circulation-type column oven, a SPD-M20 photo diode array UV/VIS detector, and LC Solution 1.2 software (Shimadzu, Tokyo, Japan). The analyses were carried out in reversed phase mode using a Phenomenex Luna C18 column (150 mm x 4.6 mm x 5 µm) maintained at 30 ºC. The mobile phase consisted of acetonitrile (A) and water (B), which were filtered prior to their use with 0.45 µm regenerated cellulose membrane filters. The mobile phase was eluted at a flow rate of 1.0 mL/min using the following gradient program: 53%-65% A at 0-12 min, and 65%-75% at 12-15 min. After this time the column was set to initial conditions in 3 min and rebalanced under these conditions for 4 min. The injection volume of the samples was 20 µL and the detection of jatrophone was monitored at 280 nm. The total runtime was 23 min and the jatrophone retention time was 7.8 min. For the analysis, 400 µl of each sample was placed into polycarbonate optical cells and subjected to rotation at 4,000 rpm for 7.650 s, at a temperature of 25 ºC and interval time of 30 s (Yuan et al., 2013). The samples were analyzed in triplicate without prior dilution. The results were displayed as space- and time-related transmission profiles over the sample and instability index.

Accelerated stability studies

The accelerated stability study of LNC$_{Ji}$ and LNC$_{blank}$ stored at 4 ºC, was performed using an analytical centrifuge (LUMiSizer, L.U.M. GmbH, Germany), which consists of a centrifugal rotor with 12 optical cells and a light source (pulsed near-infrared light-emitting 880 nm diode and a light sensor). This equipment allows the measurement of the intensity of the transmitted near-infrared light as a function of time and position over the entire sample length as the samples are simultaneously subjected to centrifugal force, thus providing information about instability phenomena, such as sedimentation, flocculation, or creaming. For the analysis, 400 µl of each sample was placed into polycarbonate optical cells and subjected to rotation at 4,000 rpm for 7.650 s, at a temperature of 25 ºC and interval time of 30 s (Yuan et al., 2013). The samples were analyzed in triplicate without prior dilution. The results were displayed as space- and time-related transmission profiles over the sample and instability index.

In vitro drug release studies

The release studies were carried out in biorelevant media using the dialysis bag method (Luo et al., 2006). Fasted state simulated gastric fluid (FaSSGF) and fasted state simulated intestinal fluid (FaSSIF-V2) were used for simulating the fasted gastric and intestinal environments, respectively (Vertzoni et al., 2005; Jantratid et al., 2008). The fed state simulated intestinal fluid (FeSSIF ‘old’) proposed by Wagner et al. (2012) was employed for simulating intestinal fed

ultrafiltration/centrifugation procedure (5000 rpm for 10 min) using an ultrafiltration device (Amicon Ultra with Ultrace1-100 membrane, 100 kDa MWL, Millipore, USA) and the supernatants were then analyzed by HPLC using the same conditions described above. EE% was estimated as being the difference between the total concentration of jatrophone found in the LNC$_{Ji}$ and the concentration found in the supernatants. All analyses were carried out in triplicate.
state conditions. To each medium, 0.2 % sodium lauryl sulfate was added to obtain the sink conditions (Table I). For the experiment, an aliquot of either the LNC$_{j1}$ or free DF$_{j1}$ was placed into a dialysis bag (MWCO 14,000, Sigma Aldrich, USA) which was then placed into a dissolution apparatus (USP 2, Varian, USA) containing 200 mL of medium per vessel. The release studies were performed at 37 °C under mechanical stirring at 75 rpm. Samples of the release medium were withdrawn at prefixed time intervals and an equal volume of release medium was immediately replaced using fresh medium. The samples were diluted with acetonitrile, filtered through 0.45 µm PVDF membranes, and analyzed using HPLC, according to the same experimental conditions described above. Calibration curves for jatrophone in the different release media were prepared over a concentration range from 0.1 or 0.3 to 15.0 µg/mL and were considered linear over the tested concentration range ($R^2 > 0.99$). LOD values were 0.02, 0.07, and 0.03 µg/mL, and LOQ values were 0.07, 0.22, and 0.10 µg/mL for FaSSGF, FaSSIF-V2, and FeSSIF ‘old’ media, respectively, indicating the method was sensitive enough to quantitate the drug release. All dissolution experiments were performed in triplicate. The cumulative percentage of jatrophone released in each medium (%) was plotted against time (h). Dissolution efficiency (DE% ± s.d.) was calculated from the area under the dissolution profile and expressed as the percentage of the area of the rectangle described by 100% dissolution within the same period of time.

Table I - Dissolution media simulating gastric (FaSSGF) and intestinal (FaSSIF-V2) fasted state conditions, and intestinal fed (FeSSIF ‘old’) state conditions.

<table>
<thead>
<tr>
<th>Composition</th>
<th>FaSSGF</th>
<th>FaSSIF-V2</th>
<th>FeSSIF ‘old’</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>1.6</td>
<td>6.8</td>
<td>5.0</td>
</tr>
<tr>
<td>Sodium taurocholate</td>
<td>80 µM</td>
<td>3 mM</td>
<td>15 mM</td>
</tr>
<tr>
<td>Lecithin from egg</td>
<td>20 µM</td>
<td>0.2 mM</td>
<td>3.75 mM</td>
</tr>
<tr>
<td>Pepsin</td>
<td>0.1 mg/mL</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>34.2 mM</td>
<td>68.62 mM</td>
<td>203.2 mM</td>
</tr>
<tr>
<td>Maleic acid</td>
<td>-</td>
<td>19.12 mM</td>
<td>-</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>-</td>
<td>34.8 mM</td>
<td>101 mM</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>qs. pH 1.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>-</td>
<td>-</td>
<td>144.1 mM</td>
</tr>
<tr>
<td>Sodium lauril sulfate</td>
<td>0.2 %</td>
<td>0.2 %</td>
<td>0.2 %</td>
</tr>
</tbody>
</table>

Pharmacological activity of LNC$_{j1}$ in a carrageenan-induced arthritis model in rats

Animals and drugs

All experiments were performed using adult male Wistar rats weighing 250–300 g. The animals were housed under a controlled temperature ($21 ± 2 ^\circ C$) on a 12 h light/12 h dark cycle with standard lab chow and water ad libitum until the experimental sessions. The animals were acclimatized into the experimental room for at least 30 min before the experiments. The experiments were conducted in accordance with the ethical guidelines of the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).
and the International Association for the Study of Pain (Zimmermann, 1983) and approved by the local committee for the ethical use of animals (P00723/CEUA-UFSC). The DF$_J$ was resuspended in a mixture composed of dimethyl sulfoxide (DMSO), polyethylene glycol 400 (PEG 400), and phosphate buffered saline (PBS), pH 7.4 (5:47.5:47.5 v/v), for oral administration (p.o.) in rats.

**Carrageenan-induced articular incapacitation in rats**

Articular incapacitation was induced by the injection of 300 µg of carrageenan (solubilized in 50 µL sterile 0.9% saline) into the right knee joint of rats. In this assay, the animals were stimulated to walk on a revolving steel cylinder (constant speed of 3 rpm) wearing metallic gaiters on the hind paws. The right gaiter was connected to a computer system that recorded the amount of time spent with that paw lifted from the cylinder surface during the one-minute test period. This paw elevation time (PET), recorded in seconds, was taken as an estimate of nociception (Tonussi, Ferreira, 1992).

2 h after carrageenan injection, the animals received treatment with either DF$_J$ (50 mg/kg) or LNC$_J$ (50 mg/kg), both administered in a single-dose oral gavage. Oral dexamethasone (10 mg/kg) was used as a positive control while the negative control groups were treated with either vehicle or LNC$_{blank}$. PET was evaluated hourly from hour three to six, and expressed as an average of these time points.

**Edema measurement**

A measurement of the articular diameter (AD) was used to quantify the inflammatory edema induced by carrageenan. AD was taken by measuring the medio-lateral axis of the knee-joint (in mm), using a micrometer, at three consecutive arbitrary points in a proximo-distal direction. 2 h after carrageenan injection, the animals were treated with either DF$_J$ (50 mg/kg) or LNC$_J$ (50 mg/kg), both administered via a single-dose oral gavage. The AD measured just before carrageenan injection was subtracted from AD values taken hourly from hour three to hour six, just after the incapacitation measurements, and values were presented as an average of these time points. Oral dexamethasone (10 mg/kg) was used as a positive control and the negative control groups were treated with the vehicle or LNC$_{blank}$.

**Statistical analyses**

Data were expressed as mean ± standard deviation (s.d.) and mean ± S.E.M. The statistical significance was assessed by $t$-test or one-way analysis of variance (ANOVA) followed by post-hoc Tukey or Dunnett’s Multiple Comparison test. P values lower than 0.05 ($P<0.05$) were considered indicative of significance. The sample size for incapacitation and articular edema were estimated using a statistical power test and, thus, a minimum of six animals were used for both parameters.

**RESULTS AND DISCUSSION**

**Analysis of fatty acids by GC-FID**

Previous studies chemically characterizing DF$_J$ obtained from the underground parts of $J$. isabellei have revealed that this extract is mainly composed of terpenic compounds, of which jatrophone has been identified as an important diterpene, constituting approximately 9 wt% of the extract (Fröhlich et al., 2017). Dichloromethane is a non-polar solvent that has been used to extract lipids and fatty acids from plants (Cequier-Sánchez et al., 2008) and, due to the fact that the concentration of these compounds could impact the preparation of LNCs, the fatty acid composition of this fraction was determined by GC-FID. The analysis of DF$_J$ demonstrated the presence of 30 total compounds, 9 of which were identified, representing 50.49% of the total fatty acid composition. The major fatty acids found in DF$_J$ were arachidic acid (C20:0; 19.82%), linoleic acid (C18:2; 12.64%), oleic acid (C18:1; 8.05%), and heneicosanoic acid (C21:0; 7.06%), followed by small amounts of linolelaidic acid (C18:2), tridecanoic acid (C13:0), myristic acid (C14:0), lauric acid (C12:0), and capric acid (C10:0). The presence of these fatty acids...
acids has also previously been found in the oil seeds of *Jatropha* genus plants, which have been studied in biodiesel production (Akbar *et al.*, 2009; Berchmans, Hirata, 2008). Here, the characterization of the DF*Ji* in terms of fatty acid composition will be useful for the development of lipid drug delivery systems, since this feature may affect the physicochemical properties and stability of the final drug products.

### Preparation, characterization, and stability evaluation of the lipid nanocarriers

In this study, unloaded (LNC$_\text{blank}$) and DF*Ji*-loaded (LNC$_\text{Ji}$) LNCs were prepared by the spontaneous emulsification method, which is a low energy method in which the very fast diffusion rate of the water-miscible organic solvent towards to the aqueous phase leads to the spontaneous formation of droplets in the submicron-size range. The physicochemical and drug loading properties of the LNCs obtained in this study are summarized in Table II. Both LNC$_\text{blank}$ and LNC$_\text{Ji}$ displayed mean size ranges from 180 to 200 nm and polydispersity indices (PDIs) lower than 0.20, indicating the formation of colloidal dispersions with monodisperse size distributions. Both LNCs exhibited negative surface charges, with zeta potential values of -19.6 mV and -18.8 mV for LNC$_\text{Ji}$ and LNC$_\text{blank}$, respectively. As can be seen, the zeta potential values were similar for both LNC$_\text{blank}$ and LNC$_\text{Ji}$. The negative values may be caused by the presence of small amounts of acidic lipids in the soybean lecithin, such as phosphatidylinositol, phosphatidylserine, and phosphatidylglycerol, which are preferentially located on the LNC surface (Schuh, Bruxel, Teixeira, 2014). In general, it has been accepted that zeta potential values higher than |30| mV are required for electrostatic stabilization of colloidal dispersions. However, non-ionic surfactants, such as polysorbate 80, are able to stabilize nanodispersions through steric effects instead of electrostatic stabilization (Kronberg *et al.*, 1990).

The pH of the formulations were 4.13 ± 0.1 and 5.25 ± 0.2 for LNC$_\text{Ji}$ and LNC$_\text{blank}$, respectively. Jatrophone was used as chemical marker to monitor the encapsulation of the DF$_\text{Ji}$ in the LNC$_\text{Ji}$, since it has been found to be an important constituent of this fraction. The EE% obtained for jatrophone was higher than 90%, which may be explained by the high lipophilicity of this bioactive compound and, therefore, its high affinity for the internal phase of the colloidal dispersion. The transmission electron microscopy images of LNC$_\text{Ji}$ negatively stained with phosphotungstic acid exhibited spherical particles with sizes around 100 - 200 nm (Figure 1).

### Table II - Physicochemical and loading properties of the lipid nanocarriers.

<table>
<thead>
<tr>
<th></th>
<th>Mean droplet size (nm) (PDI)$^1$</th>
<th>Zeta potential (mV)</th>
<th>pH</th>
<th>Jatrophone content (µg/mL)</th>
<th>Entrapment efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LNC$_\text{blank}$</td>
<td>191.1 ± 4.6 (0.15)</td>
<td>-18.8 ± 4.4</td>
<td>5.25 ± 0.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LNC$_\text{Ji}$</td>
<td>182.9 ± 2.7 (0.09)</td>
<td>-19.6 ± 1.8</td>
<td>4.13 ± 0.1</td>
<td>867.2 ± 11.1</td>
<td>90.4 ± 0.9</td>
</tr>
</tbody>
</table>

$^1$Polydispersity index. The results were expressed by mean ± s.d (n=3).
The high stability of LNC$_{Ji}$ stored at 4 °C was evidenced through the accelerated stability testing using the analytical centrifuge (LUMiSizer®). Figure 2 illustrates the representative light transmission profiles averaged over the height of the sample for unloaded (LNC$_{blank}$) and DF$_{Ji}$-loaded lipid nanocarriers (LNC$_{Ji}$). The light transmission profiles of the LNC$_{blank}$ revealed a clarification area (an increase in the light transmission) at the bottom of the cuvette, possibly due to an initial creaming phenomenon. The colloidal dispersion stability can also be described by the instability index values. In this assay, the instability index obtained for LNC$_{blank}$ was 0.536 ± 0.083, while LNC$_{Ji}$ exhibited an instability index of 0.083 ± 0.004. The lower instability index obtained for LNC$_{Ji}$ indicates that the presence of the constituents of DF$_{Ji}$ contribute to the physical stabilization of the colloidal dispersion, probably due to the presence of fatty acid lipid compounds.
**In vitro drug release studies**

It is well known that two of the main processes influencing the absorption of orally administered drugs are: the dissolution of drug in the biological fluids at the site of absorption, and the transport of the drug to the systemic circulation through the gastrointestinal mucosa (Wagner et al., 2012). In this way, drug release studies using biorelevant media are an interesting approach for predicting the *in vivo* performance of lipid oral dosage forms, as such media better simulates the physiological conditions present in the stomach and small intestine. Since jatrophone has been found to be an important compound of DF$_{Ji}$-loaded LNCs, we performed our release studies by the measuring the concentration of this diterpene in the FaSSGF, FaSSIF-V2, and FeSSIF ‘old’ media. The jatrophone solubility was previously determined in each media and the amount used for these
studies followed the sink conditions. In this study, the release profile of jatrophone from LNC$_{ji}$ was compared to the release profile of free DF$_{ji}$ dispersed directly in the release media.

The jatrophone release profiles are shown in Figure 3. In FaSSGF media, the release of jatrophone from LNC$_{ji}$ reached 38.81% by 2 h, compared to only 23.4% of this diterpene released from DF$_{ji}$ in the same amount of time. The cumulative percentages of released drug for the free DF$_{ji}$ and LNC$_{ji}$ assays were 69.3% and 86.6% in FaSSIF-V2 media, and 61.0% and 104.2% in FeSSIF ‘old’ media, respectively. To compare the jatrophone release profiles obtained for LNC$_{ji}$ and free DF$_{ji}$, the dissolution efficiency values (DE%) were calculated (Table III). The results demonstrate that the release rates of jatrophone from LNC$_{ji}$ and free DF$_{ji}$ suspension were statistically different in FaSSGF and FeSSIF media ($P \leq 0.001$), but were similar in FaSSIF medium.

This study clearly demonstrates the ability of LNCs to carry DF$_{ji}$ in a very fine aqueous dispersion, thus increasing the release rate of lipophilic drugs such as jatrophone. The differences found in the rates of jatrophone release reflect not only the pH, buffer capacity, and osmolarity of the biorelevant media, but also the presence of physiological surface active species, such as bile salts and phospholipids. Particularly for lipophilic drugs, such as those belonging to BCS class II, dissolution behavior has been demonstrated to be strongly influenced by the presence of these natural surfactants (Galia et al., 1998). According to the release profiles, the percent of jatrophone released from LNC$_{ji}$ after 2 h in FaSSGF was almost twice the amount released from the free fraction. In FeSSIF ‘old’ media, the large amount of emulsifying components (15 mM sodium taurocholate and 3.75 mM egg lecithin) contributed to the higher solubilization of jatrophone, thus increasing the jatrophone release rate, especially from LNC$_{ji}$ as the amount of drug released was approximately 40% higher than that from the free fraction. On the other hand, the similarity between the release profiles in FaSSIF-V2 media may be explained by the solubility of jatrophone in this media, due to the combination of the presence of lecithin and sodium taurocholate with the pH, which favors the solubilization of jatrophone from the free fraction.
FIGURE 3 - *In vitro* jatrophone release (± s.d.) from LNC\textsubscript{Ji} and free DF\textsubscript{Ji} in (A) fasted state simulated gastric fluid (FaSSGF) media, (B) fasted state simulated intestinal fluid (FaSSIF) media, and (C) fed state simulated intestinal fluid (FeSSIF), all at 37 °C (n=3).
**Table III** - Dissolution efficiency values (DE%) calculated from the jatrophone release profiles.

<table>
<thead>
<tr>
<th></th>
<th>FaSSGF Free DF&lt;sub&gt;i&lt;/sub&gt;</th>
<th>FaSSGF LCN&lt;sub&gt;i&lt;/sub&gt;</th>
<th>FaSSIF-V2 Free DF&lt;sub&gt;i&lt;/sub&gt;</th>
<th>FaSSIF-V2 LCN&lt;sub&gt;i&lt;/sub&gt;</th>
<th>FeSSIF ‘old’ Free DF&lt;sub&gt;i&lt;/sub&gt;</th>
<th>FeSSIF ‘old’ LCN&lt;sub&gt;i&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>DE (%)</td>
<td>16.0* ± 0.5</td>
<td>26.8* ± 0.7</td>
<td>42.6 ± 5.6</td>
<td>50.2 ± 1.3</td>
<td>38.1± 3.0</td>
<td>63.8± 2.4</td>
</tr>
</tbody>
</table>

The symbols * and # denotes statistical difference at $P<0.001$.

**Pharmacological activity of LNC<sub>i</sub> in a carrageenan-induced arthritis model in rats**

The effects of oral administration of either free DF<sub>i</sub> or LNC<sub>i</sub> at a dose of 50 mg/kg is demonstrated in Figure 4. The animals that received only vehicle or LNC<sub>blank</sub> p.o. 2 h after carrageenan knee-joint injections (negative control groups) exhibited average PET values that increased from 9.4 ± 0.4 and 9.7 ± 0.7 sec at basal levels to 31.4 ± 1.8 sec and 30.3 ± 2.0 sec, respectively (Figure 4). Animals receiving treatment with free DF<sub>i</sub> did not exhibit a significant reduction in average PET values compared to the negative control groups, demonstrating a value of 27.9 ± 1.0 sec, consistent with previous studies (Fröhlich et al., 2017). However, the animals treated with LNC<sub>i</sub> at the same dose demonstrated a significant reduction in their average PET values, with a value of 20.2 ± 0.5 sec ($P<0.001$). The effects of DF<sub>i</sub> administration on PET were compared to the oral administration of dexamethasone (10 mg/kg), a drug which is commonly used to treat arthritis. The administration of this drug caused a significant reduction in the average PET values when compared to the negative control groups, with an average value of 13.0 ± 0.3 sec. These findings could be explained, at least in part, by the ability of LNC<sub>i</sub> to increase the oral absorption of the DF<sub>i</sub> constituents solubilized in the oil phase of the colloidal dispersion, especially the diterpene jatrophone, which is thought to be related to the antinociceptive properties of the substance via its capacity to inhibit [$^3$H] glutamate binding in a dose-dependent way (Martini et al., 2000). These results are in accordance with the in vitro release studies, which indicated faster release rates for jatrophone from LNC<sub>i</sub> in biorelevant media, pointing towards the idea that the dissolution of DF<sub>i</sub> constituents is a rate-limiting step in their absorption via the gastrointestinal tract.

In contrast, oral administration of LNC<sub>i</sub> did not significantly reduce the knee-joint edema in rats compared to the administration of free DF<sub>i</sub> at the same dose (data not shown). In this case, the increased absorption of DF<sub>i</sub> could also be reverting the antiedematogenic effect, due to a reported vasodilatory effect of jatrophone. Such vasodilatory effects from jatrophone have been demonstrated on the portal vein and aorta, where they were shown to be related to the inhibition of PKC-dependent mechanisms (Silva, Brum, Calixto, 1995) and to the blockade of Ca$^{2+}$ influx through voltage-sensitive channels and activation of K$^+$ channels (Duarte, Sant’Ana, Calixto, 1992), respectively. Considering the results of our in vivo studies, the beneficial effects observed from the popular use of the *J. isabellei* plant to treat arthritis may be, at least in part, related to the antinociceptive properties of its terpene compounds. In this case, the encapsulation of DF<sub>i</sub> into LNCs seems to improve the absorption of the lipophilic constituents of the DF<sub>i</sub> fraction, thus presenting great potential for the treatment of arthritic conditions.
Jatropha isabellei terpenes loaded into oral nanoemulsions

FIGURE 4 - Effects of oral administration of LNC\textsubscript{Ji} and DF\textsubscript{Ji} on paw elevation time (PET). The animals were treated 2 h after an intra-articular injection of carrageenan (300 µg/knee). The negative control group received vehicle (DMSO:PEG 400:PBS, 5:47.5:47.5) or LNC\textsubscript{blank}. Dexamethasone (DEX, 10 mg/kg, p.o.) was used as a positive control. ***P<0.001 represents a significant difference compared with the vehicle and LNC\textsubscript{blank}. The statistical analysis was performed using one-way ANOVA followed by Dunnett’s post hoc test.

CONCLUSIONS

LNCs containing constituents of the dichloromethane fraction dry extract from underground parts of \textit{J. isabellei} (DF\textsubscript{Ji}) were successfully obtained using the spontaneous emulsification method. These LNC\textsubscript{Ji} exhibited a spherical shape, monodisperse size distribution, and high entrapment efficiency of jatrophone. The stability of the LNCs stored at 4 °C was demonstrated through accelerated stability testing. Studies on the drug release in biorelevant media showed that jatrophone was released from LNC\textsubscript{Ji} at a faster rate in FaSSFG and FeSSIF ‘old’ than from free DF\textsubscript{Ji}. Additionally, our pharmacological studies in a carrageenan-induced arthritis model demonstrate that the oral administration of 50 mg/kg LNC\textsubscript{Ji} exhibited antinociceptive properties demonstrated by significantly reduced PET values. This was in contrast to administration of free DF\textsubscript{Ji}, which was not able to reduce the PET values. However, no reduction in AD was observed when LNC\textsubscript{Ji} was administered at the same dose as DF\textsubscript{Ji}. These results suggest that loading DF\textsubscript{Ji} into LNCs may improve the oral absorption of the active constituents of this lipophilic fraction by overcoming its poor water solubility, thus improving the compound’s antinociceptive properties and resulting in more beneficial pain relief.

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