Saudi Pharmaceutical Journal 27 (2019) 191–199

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

Cassia grandis Lf nanodispersion is a hypoglycemic product with a potent α-glucosidase and pancreatic lipase inhibitor effect

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ARTICLE INFO

Article history:
Received 30 March 2018
Accepted 16 October 2018
Available online 17 October 2018

Keywords:
Hypoglycemic
Nanodispersion
Cassia grandis
Lipase inhibitory
Alpha-glucosidase
Antioxidant

ABSTRACT

Purpose: This study aimed to evaluate the hypoglycemic effect, antioxidant, α-glucosidase and lipase inhibitory activity, and the cytotoxicity of the *Cassia grandis* nanodispersion (CgND).
Methods: The hypoglycemic effect was evaluated in alloxan-induced diabetic mice. The particle size, polydispersion index, ζ-potential, and conductivity, as well as the drug-loaded content, were monitored in shelf-live, along a year. The delivery profile was evaluated in simulated intestinal fluids at pH 6.5 and 7.4. The antioxidant effect was evaluated as DPPH and ABTS inhibition. The murine α-glucosidase inhibitory activity and the lipase-inhibitory effect were evaluated *in vitro.* Cytotoxicity was evaluated by the Alamar blue test.
Results: CgND remained stable for a year in shelf conditions. The hypoglycemic effect in a dose of 10 mg/kg was not statistically different from glibenclamide 25 mg/kg. Nanoparticles released 100% of extract in 120 min at pH 6.5 and 7.4. Nanodispersion exhibited a potent α-glucosidase and lipase-inhibitory effect with IC_{50} of 3.96 and 0.58 μg/mL, respectively. A strong antioxidant activity against DPPH (IC_{50} 0.65 μg/mL) and ABTS (0.48 μg/mL) was also observed. The hypoglycemic effect could occur, at least in part, via antioxidant and α-glucosidase inhibition. CgND is non-cytotoxic in MRC-5 line cell. This nanodispersion is a promising nanotechnological product that could be used in pharmaceuticals for the treatment of Type II diabetes and related complications as obesity.

1. Introduction

Diabetes mellitus is the first non-transmitted diseases declared as epidemy by the World Health Organization (WHO, 2016). Clinical studies revealed hyperglycemia as the leading cause of coronary disease, cerebrovascular, renal failure, limb amputation, and lipid abnormalities (Daisy and Saipriya, 2012). Despite the progress of the diabetes management, controlling its lethal consequences is extremely difficult. In 2014, there were 422 million of diabetics around the world. In the USA one in seven adults has diabetes, in Brazil there is one diabetic in every six people (WHO, 2016). WHO is calling for action on diabetes prevention and treatment because it has become a global health problem and efforts for developing new antidiabetic drugs are still insufficient.

The use of medicinal plants for diabetic people has increased, because of the necessity for controlling blood glucose levels. Populations of the Caribbean, Lesser Antilles, and Central America informed the use of *Cassia grandis* Lf in diabetes (Ezuruike and Prieto, 2014). Despite the pungent smell and a particularly strong

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jsps.2018.10.003
1319-0164/

flavor of this plant fruit, the pulp is routinely consumed as a fresh drink for reducing the blood glucose level. However, there are no reports on the antidiabetic effect *in vivo* or *in vitro* of the fruit nor of its extracts (Lafourcade et al., 2018).

Develop an active dosage form using botanical extracts is a challenge. In most cases they are water-insoluble, presenting low bioavailability, and unstable against factors such as light, oxygen, and temperature. Usually, the systemic clearance of compounds presents in vegetal extracts increases, needing higher doses, making herbal products poor therapeutic candidates (Ansari et al., 2012). *C. grandis* fruit pulp contains triterpenes and steroids, essential oils, reducing sugars, amino acids, and amines, saponins, phenol and tannins, flavonoid and coumarins (Lafourcade et al., 2014). Hydroalcoholic extracts prepared with the fruit pulp are light sensitive and easily oxidized because of the presence of coumarins, flavonoids, phenolic, and reducing sugar.

Pharmaceutical preparations obtained by nanotechnological approaches can overcome pharmacokinetics, bioavailability, and stability problems of botanical extracts (Ansari et al., 2012). The integration of nanotechnology techniques with the traditional way for preparing botanical products could be essential for obtaining pharmaceutical preparation for using in the treatment of chronic diseases like asthma, diabetes, and cancer (Yadav et al., 2011).

The small size and architecture of nanoparticles produce a significant increase in surface area, improving the drug activity *in-vitro* and *in-vivo*, as compared with the original drug (Wu et al., 2011). Additionally, the complex composition of botanical extracts suggests the occurrence of multiples mechanism of action that, combined with the advantage offered by nanotechnology, become nanoparticles prepared with vegetal extracts in excellent therapeutic candidates to treat multifactorial cause diseases such as diabetes. In the present work, the hypoglycemic effect of the *C. grandis* nanodispersion was assayed in alloxan-induced diabetic mice. Additionally, the release profile, stability, antioxidant activity, and the inhibitory effect of α-glucosidase and lipase were also evaluated.

2. Materials and methods

2.1. Plant material and extract preparation

The fruits of *C. grandis* were collected in El Caney, Santiago de Cuba, Cuba (Latitude: 20.0569, Longitude: - 75.7719) in April 2015. Felix Acosta Cantillo made the plant material identification, and a voucher with registration number 1965 is deposited in the BIOECO herbarium, Santiago de Cuba, Cuba.

The extract was prepared by maceration (72 h) from the fresh fruits pulp (1 kg), using 70% hydroalcoholic solution (2 L). The extract was concentrated using a rotary evaporator at 40 °C (KIKA WERKE GMBH & Co. Germany), at a final drug: solvent ratio of 2:1 (w/v) (Lafourcade et al., 2018).

2.2. Nanoparticles preparation

Nanodispersion was prepared by the interfacial polymer deposition method followed by solvent displacement, as was described in a previous work (Lafourcade et al., 2016).

2.3. Dynamic light scattering (DLS)

Particle size and the polydispersion index were measured by DLS, using a Zetasizer (Malvern, UK). Nanodispersion was diluted 1:9 (v/v) in water for injection (Ω < 4 μS) and filtered by a Millipore® membrane (0.45 μm). Measures were made in a square glass cuvette, using a laser wavelength of 633 nm, 173° of scattering angle, at 25 °C. The equipment was stabilized for 30 min before the use (Malvern, 2015). Measurements were made in triplicate and results were expressed as the mean ± standard deviation.

2.4. ζ-potential

The ζ-potential and conductivity were measured by Doppler Laser Micro-electrophoresis in a Zetasizer (Malvern, UK), using a voltage of 150 V. For the measure, the nanodispersion was dissolved in water for injection 1:10. Measurements were made at 25 °C and results were expressed as the mean ± standard deviation (n = 3).

2.5. Entrapped efficiency by HPLC

High-performance liquid chromatography was used to determine the amount of the extract encapsulated, expressed as pyrogallic acid. One ml of the nanodispersion was diluted with 4 mL of water for injection and vortexed for 1 min. The solution was centrifuged at 13,000 rpm (Sigma, Germany), for 15 min, at 5 °C. The supernatant (1 mL) was filtered (Millipore® 0.22 μm), and 200 μL was dissolved in 800 μL of a methanol: water (1:1) solution. The analysis was made in an HPLC system (Shimadzu, Japan), using a diode array detector at 330 nm, at 30 °C. A reverse phase column Phenomenex Luna® C18 (2), (250 × 4.6 mm, 5 μm) was used with an injection volume of 10 μL. The analysis was made in an isocratic system using methanol (phase A, 70%) and 0.1% of acetic acid solution (Phase B, 30%), with a flow rate of 1 mL/min. The runtime was 15 min. Determinations were made by the calibration curve method using pyrogallic acid (Sigma, USA) standard solution of 4, 8, 16, 32, and 64 μg/mL of concentration. The assay was made in triplicate, and the mean ± standard deviation was reported. The encapsulation efficiency (EE) was calculated by the expression (1).

$$EE\ (\%) = [(PCgE - PS) \times 100]/PCgE \quad (1)$$

where PCgE, phenolics in the pure extract; PS, phenolics in the supernatant liquid.

2.6. Stability study

2.6.1. Drug-loaded nanoparticles

Nanodispersion was stored in a glass flask for a year at 25 ± 2 °C, 60 ± 5% of relative humidity, protected from light. The amount of extract entrapped in nanoparticles was verified every two months, repeating the procedure described in Section 2.5. The assay was made in triplicate, and the mean ± standard deviation was reported.

2.6.2. Particle size, zeta potential, and conductivity

Particle size and polydispersion index were measured every two months along a year, as was described in Section 2.3. Zeta potential and conductivity were measured as was described in Section 2.4. Both assays were made in triplicate, and the mean ± standard deviation was reported.

2.6.3. pH

The pH of the nanodispersion was measured every two months, using a pH-meter (Tecnopon, Brazil), previously calibrated with buffer solutions of pH 4, 7, and 10 (Alphatech, Brazil), with a precision of 0.02 units. The assay was made in triplicate, and the mean ± standard deviation was reported.

2.7. Delivery profile

The release profile *in vitro* was evaluated in simulated intestinal fluid (SIF; pH 6.5) and simulated intestinal fluid without pancreatin (SIFwP; pH 7.4) (Rodriguez et al., 2016). The dialysis-bag diffusion method was used. CgND (20 mL) was placed in a cellulose membrane dialysis bag perfectly sealed. The bag was immersed in 100 mL of each media at 37 ± 0.5 °C, and the vessel was stirred at 200 rpm. Aliquots (3 mL) of solution were withdrawn at times 0, 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, and 120 min, and replaced by new solution media. The amount of drug released was determined by HPLC as was described in Section 2.5.

2.8. Biological activity in vitro

2.8.1. DPPH radical scavenging

The radical scavenging activity was determined by the Burits and Bucar method (2000) with slight modifications. Equal volumes of DPPH ethanolic solution (0.05%) and different dilutions (0.013, 0.026, 0.052, 0.103, 0.206, 0.412, 0.825, 1.650 μg/ml) of CgE and CgND in distilled water were incubated in the dark, at room temperature, for 30 min. Gallic acid (1 mg/mL) and dimethylsulfoxide (DMSO) were used as a positive and negative control, respectively. The absorbance diminution was measured at 492 nm using a microplate detector (DTX 800, Beckman, UK). The scavenging activity (in percent) was calculated using the following formula:

$$\%\ \text{inhibition} = 100 - [\text{Abs}_{a/p}/\text{Abs}_C] \times 100 \quad (2)$$

where: $\text{Abs}_{a/p}$ = (Absorbance of Blank − Absorbance of Sample)

Abs_C = (Absorbance of Blank − Absorbance of the Control)

The experiment was made in triplicate. Results were expressed as the means ± standard deviation. The IC_{50} was determined by Probit analysis using the GraphPad Software (CA, USA), at a significance level of 0.05.

2.8.2. ABTS assay

The assay was made by the Shanty's method (2017) with some modifications. The working solution was prepared by mixing an ABTS stock solution (7 mM) and potassium persulfate solution (2.4 mM) in a ratio 1:1. The mixture was incubated 14 h in the dark, at room temperature. After that, it was diluted with distilled water (1:5). For the assay, 30 μL of CgE and CgND solutions of different concentrations (0.013, 0.026, 0.052, 0.103, 0.206, 0.412, 0.825, 1.650 μg/ml) were incubated for 15 min in the dark, at room temperature, with 270 μL of the working solution. The blank was composed of water (270 μL) plus 30 μL of the nanosuspension. Gallic acid (1 mg/mL) was used as a positive control and (DMSO) as a negative control. The absorbance was measured at 620 nm in a microplate detector (DTX 800, Beckman, UK). All determinations were performed in triplicate (n = 3). The results were expressed as percent of inhibition and were calculated as:

$$\%\ \text{inhibition} = 100 - [\text{Abs}_{a/p}/\text{Abs}_C] \times 100 \quad (3)$$

where: $\text{Abs}_{a/p}$ = (Absorbance of Blank − Absorbance of Sample)

Abs_C = (Absorbance of Blank − Absorbance of the Control)

The IC_{50} was determined by Probit analysis using the GraphPad Software (CA, USA), at 0.05 level of significance.

2.8.3. Lipase inhibitory activity

The assay was performed according to Slan et al. (2009) with slight modifications. Porcine pancreatic type II lipase (code L3126-25G, Sigma, USA) was diluted in TRISMA-HCl pH 8.5 buffer, at a concentration of 0.8 mg/mL. The substrate 4-nitrophenyl palmitate (PNP), code N2752-50G (Sigma, USA) was diluted in acetonitrile: ethanol at a concentration of 4 mg/mL. In different microplate wells, were placed 30 μL of CgE and CgND of different concentrations, Orlistat (Sigma, USA) solution, and DMSO with 250 μL of the lipase solution. The microplates were incubated for 5 min at 37 °C in the dark. Then, 20 μL of the PNP solution was added. The mixture was incubated for 10 min until the control absorbance at 450 nm was equal to 1000 ± 0.1. A microplate reader (DTX 800, Beckman, UK) was used. The analysis was performed in triplicate, and the percent inhibition (% I) was calculated using the formula:

$$\%\ I = 100 - [\text{Abs}_{a/p}/\text{Abs}_C] \times 100 \quad (4)$$

where: $\text{Abs}_{a/p}$ = (Absorbance of Blank − Absorbance of Sample) at 450 nm

Abs_C = (Absorbance of Blank − Absorbance of the Control)

The IC_{50} was determined by Probit analysis using the GraphPad Software (CA, USA), at 0.05 level of significance.

2.8.4. Murine α-glucosidase inhibitory activity in vitro

The assay was made according to Andrade et al. (2008), with slight modifications. The release of 4-nitrophenol from 4-nitrophenyl α-D-glucopyranoside (4-NPGP) was determined. An enzymatic solution was prepared by dilution of 3 mg of intestinal acetone powders (Sigma, USA) in 1 mL of 0.1 M sodium phosphate buffer pH 6.8. This mixture was vortexed for 1 min and centrifuged at 13,000 rpm, for 10 min. The supernatant enzyme solution was separated and used immediately. A solution of 4-NPGP (5 mg/mL) in phosphate buffer pH 6.9 was also prepared. In microplate wells, were placed 30 μL of the standard solution (Acarbose, 100 μg/mL), the control (DMSO) and the CgE and CgND in concentrations of 2.5, 5, 10, 15, 20, 25, 30 and 33 μg/mL, with 170 μL of the enzymatic solution. Microplates were incubated for 5 min at 37 °C, in the dark. After that, 100 μL of 4-NPGP was added to each well and incubated for additional 20 min and/or the control absorbance at 405 nm was equal to 1.000. The percent of inhibition (% I) were calculated and samples with % I ≥ 50 were used for estimating the IC_{50} value. A microplate reader (DTX 800, Beckman, UK) was used. The analysis was performed in triplicate, and the percent inhibition (% I) was calculated using the formula:

$$\%\ I = 100 - [\text{Abs}_{a/p}/\text{Abs}_C] \times 100 \quad (5)$$

where: $\text{Abs}_{a/p}$ = (Absorbance of Blank − Absorbance of Sample) at 405 nm

Abs_C = (Absorbance of Blank − Absorbance of the Control)

The IC_{50} was determined by Probit analysis using the GraphPad Software (CA, USA), at a level of significance of 0.05.

2.9. Hypoglycemic effect in vivo

2.9.1. Animals

Swiss mice, males, of eight-weeks old and body weight between 25 and 30 g, were used. Animals were supplied by the Drug Research Laboratory, Federal University of Amapá, Brazil. They were acclimatized to laboratory controlled conditions, 25 ± 2 °C; relative humidity 60 ± 10%, a 12/12 h light/dark cycle. Animals were fed, *ad-libitum,* with standard chow (NUVILAB MCP 689, Brazil) and distilled water.

2.9.2. Diabetes induction

Diabetes was induced injecting (i.p.) 150 mg/kg of Alloxan monohydrate (Sigma, USA), every 72 h until completing three administrations. Seven days after the last dose, afterward a fasted period (12 h), the blood glucose was determined using a standard glucometer (Roche, Germany). Animals with blood glucose above 300 mg/100 mL were used for the experiment (Serreze et al., 2000, Aizman et al., 2010).

2.9.3. Hypoglycemic test

For the test, five groups of five animals each were randomly formed:

Group I: non-diabetic animals, received one ml of distilled water.
Group II: untreated diabetic mice, received one ml of distilled water.
Group III: diabetic animals treated with glibenclamide 25 mg/kg.
Group IV diabetic animals treated with *C. grandis* extract, 200 mg/kg.
Group V, diabetic animals treated with nanoparticles solution, 10 mg/kg.

All the treatments were administered daily, using a gastric cannula (Vigon, France) during 25 days. The experiment was done according to the Standard Ethics Committee for the Use of Laboratory Animals, Federal University of Amapá, Brazil; Protocol Number 012/2017. The food and water intake were daily recorded, while the body weight was determined every five days. The day 25th, animals were anesthetized with ethyl ether, and blood samples were collected from the tail vein, and the blood glucose was determined using a standard glucometer (Roche, Germany).

2.10. Cytotoxicity assay

The Alamar Blue® assay (Ahmed et al., 1994) was used to evaluate the relative cytotoxicity of the CgE and CgND. The assay was made in non-neoplastic human lung fibroblast cell (MRC-5, ATCC-USA). MRC-5 cells were seeded into 96-well flat-bottom plates (0.5×10^4 cells/well), and cultured for 24 h. All samples were dissolved in DMSO and diluted in a high glucose culture media (DMEM) at concentrations of 100, 50, 25, 12.5, 6.25, 3.125 e 1.56 μg/mL. The DMSO final concentration was less than 0.2%. The cultured cells were exposed to CgE and CgND solutions, incubating for 24 h at 37 °C in a CO_2 atmosphere (5%). The fluorescence was measured in a microplate reader (Beckman e Coulter, UK). The grown cellular was used as positive control. DMSO 0.1% was used as negative control. The reference drug (doxorubicin 5 μg/mL, Sigma-Aldrich, Brazil) was used as a cellular dead control. All experiments were made in duplicate and repeated thrice.

2.11. Data analysis

Statistical analysis was made using Statgraphics Centurion XVI.1 (Stat Easy, MA, USA). An ANOVA test followed by Tukey's HSD test was made to evaluate statistical differences among the groups, in the hypoglycemic assay. It was considered significant statistical difference for $p < 0.05$.

3. Results

3.1. Nanoparticles characterization

Fig. 1 shows the particle size and particle size distribution (Fig. 1A), and ζ-potential (Fig. 1B) of the *C. grandis* nanodispersion, 24 h after the preparation. The mean droplet size was 106.50 ± 0.44 nm with a polydispersion index of 0.124 ± 0.017. ζ-potential was equal to −9.62 ± 0.73 mV.

Nanodispersion presented a translucent aspect (Fig. 2), without aroma and tasteless, the encapsulation efficiency was 85.12 ± 0.64%.

3.2. Stability study

3.2.1. Particle size and ζ-potential stability

Fig. 3 shows the nanodispersion particle size, polydispersion index, and ζ-potential, along a year, stored at 25 °C.

3.2.2. Drug-loaded nanoparticle, pH, and conductivity

Table 1 shows the behavior of nanoparticle contain, pH and conductivity of the *C. grandis* nanodispersion along a year, stored at 25 ± 2 °C, protected from light.

The amount of extract entrapped inside nanoparticle remained without changes along the time, with no statistical differences ($p > 0.05$, Table 1). Nanodispersion pH and conductivity showed similar behavior. No statistical differences among the pH and conductivity values were observed along a year ($p > 0.05$, Table 1).

3.3. Release profile

In SIF (pH 6.5) nanoparticles released 80% in 60 min (Fig. 4), releasing the other 20% up to 120 min. On the other hand, in SIFwP (pH 7.4), 94% of the extract was released in 60 min. No statistical difference ($F = 3.56$; $p = 0.8597$) was observed between the amount of extract released in both media onwards 90 min (100%).

3.4. Antioxidant, α-glucosidase and lipase inhibitory activity

Table 2 presents the antioxidant activity as IC_{50} values of the *Cassia grandis* extract and *Cassia grandis* nanodispersion for the DPPH and ABTS assays, and for the α-glucosidase and pancreatic lipase inhibitory activity.

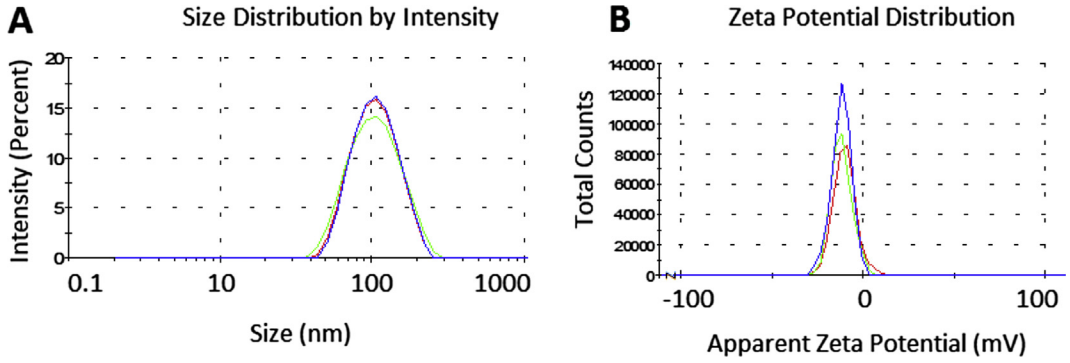

Fig. 1. Particle size (A) and ζ-potential (B) of the *Cassia grandis* nanodispersion. Mean droplet size 106.50 ± 0.44 nm, polydispersion index 0.124 ± 0.017, and ζ-potential −9.62 ± 0.73 mV. (n = 3).

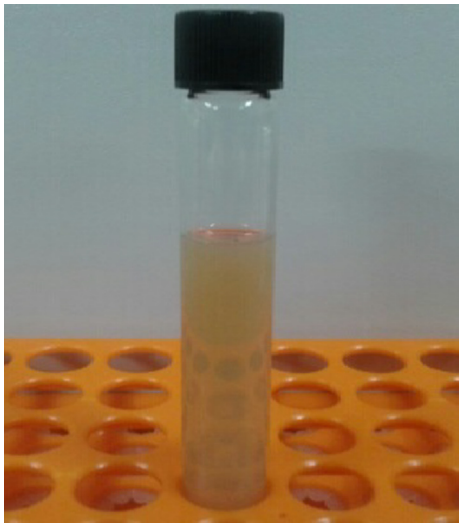

Fig. 2. *Cassia grandis* nanodispersion.

3.5. Hypoglycemic effect

Fig. 5 shows the food and water intake (Fig. 5A) and the body weight (Fig. 5B) of the five experimental groups. All groups consumed water and food normally, except the untreated diabetic group (Group II), which consumed significantly less food and drank more water. In the same way, the body weight of all the groups augmented, except the untreated diabetic group, which showed a body weight loss from 27.26 ± 1.34 to 24.06 ± 1.24 g (−11.44%; ~3.20 g).

Fig. 6 shows the effect of different treatments in the animals' blood glucose levels during the hypoglycemic activity assay.

Along the study, the blood glucose of the control group (I) kept between 85 and 105 mg/100 mL. The Group II showed a progressive increase in blood glucose. Contrarily, group III, group IV, and group V showed a progressive decrease in blood glucose levels (Fig. 6). The ANOVA test showed statistical differences among the five groups ($F = 192.54$; $p = 0.0000$). Tukey's HSD test showed that the group I and group II were different between them, and both were different from the groups III, IV, and V, which formed a homogeneous group.

3.6. Cytotoxicity

Fig. 7 presents the viability of non-neoplastic human lung fibroblast (MRC-5 cells) against different concentrations of CgE and CgND.

4. Discussion

The interfacial polymer deposition followed by solvent displacement is one of the low-energy methods using for nanoparticles preparation. In this method, the energy associated with nanoparticle formation comes from physicochemical processes rather than the application of mechanical forces (Komaiko and McClements, 2015). This fact means that no expensive equipment will be necessary for preparing the nanodispersion in large-scale. In addition, variables for increasing the production scale, using low energy input methods are usually linear (Solans and Solé, 2012). Thus, the use of an adequate polymer and solvent (Kollicoat® MAE 100P, and ethanol) and the preparation using a low

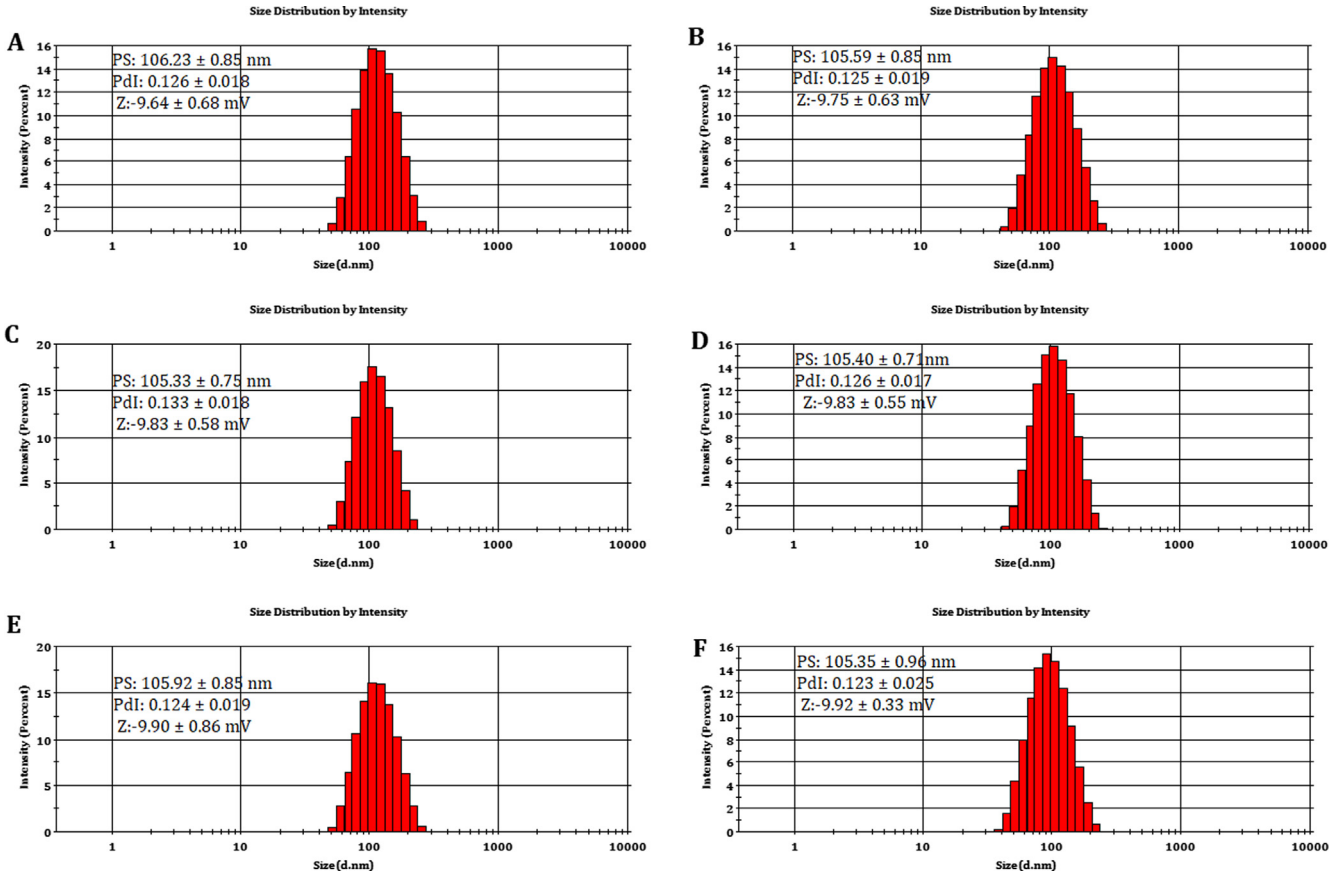

Fig. 3. Particle size (PS), polydispersion index (PdI), and ζ-potential of the *Cassia grandis* nanodispersion measured every two months (A–F), along a year.

Table 1
Extract-loaded nanoparticle, pH, and conductivity of the *C. grandis* nanodispersion along a year.

Time (month)	DLN (%)	pH (at 25 °C)	Conductivity (μS/cm)
0	85.12 ± 0.64	4.65 ± 0.02	1.42 ± 0.04
2	84.24 ± 1.10	4.63 ± 0.05	1.33 ± 0.05
4	83.70 ± 1.49	4.63 ± 0.05	1.30 ± 0.06
6	83.86 ± 2.15	4.60 ± 0.08	1.36 ± 0.04
8	83.91 ± 2.70	4.58 ± 0.10	1.37 ± 0.05
10	83.62 ± 1.49	4.61 ± 0.04	1.27 ± 0.03
12	84.37 ± 2.03	4.61 ± 0.05	1.28 ± 0.04
ANOVA	F = 0.35, p = 0.8958	F = 0.41, p = 0.8580	F = 1.76, p = 0.2372

DLN, *extract-loaded nanoparticle*, Statistical differences at $p < 0.05$.

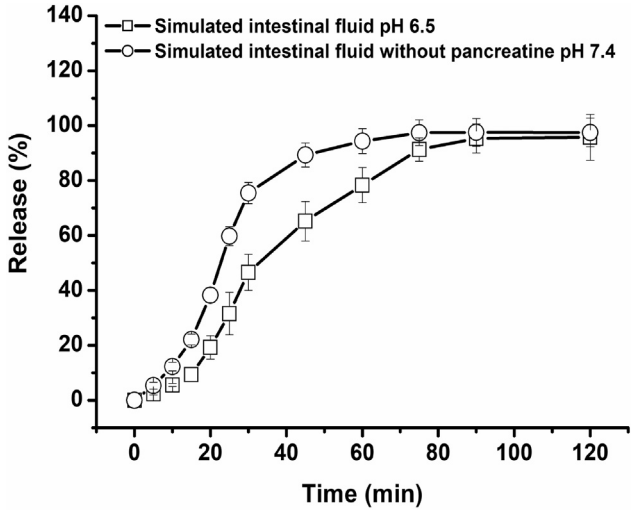

Fig. 4. Release profiles of *Cassia grandis* L f nanodispersion in simulated intestinal fluid (pH 6.5) and simulated intestinal fluid without pancreatin (pH 7.4).

Table 2
IC_{50} values of the *Cassia grandis* extract and *Cassia grandis* nanodispersion for the DPPH and ABTS assays, and for the α-glucosidase and pancreatic lipase inhibitory activity.

Assay	IC_{50} (μg/mL)		
	CgND	CgE	Control
DPPH	[a]0.65 ± 0.02	[b]1.30 ± 0.04	[c]2.90 ± 0.06[a]
ABTS	[a]0.48 ± 0.01	[b]0.72 ± 0.04	[c]2.13 ± 0.06[a]
Lipase inhibition	[a]0.58 ± 0.02	–	[b]0.29 ± 0.01[b]
α-glucosidase inhibition	[a]3.96 ± 0.5	[b]32.30 ± 1.30	[c]62.30 ± 1.30[c]

Different letters in a row mean statistical differences at $p < 0.05$.
[a] Gallic acid.
[b] Orlistat.
[c] Acarbose.

energy input process is advantageous for a further nanoparticle production in large-scale, with lower cost.

CgND showed a small particle size (106.5 nm) with narrow size distribution (PdI = 0.124), and ζ-potential −9.62 ± 0.73 mV (Fig. 1A). The negative value of zeta potential could be associated with conjugated bases from dissociates organic acids present in the non-encapsulated extract (Dias et al., 2014, Lafourcade et al., 2016). The preparation process and the good performance of the nanoparticles obtained were described in detail in a previous paper (Lafourcade et al., 2016).

The encapsulation efficiency (EE) represents the amount of active substance loaded into the nanoparticle. The amount of drug loaded depends not only on the preparation method but also on polymer–drug affinity. Some formulation variables, such as polymer-drug ratio and the type and nature of the stabilizing agents will affect the amount of drug loaded (Dupeyrón et al., 2013). The EE in CgND was 85.12 ± 0.64%. Encapsulation efficiency above 70% suggests an efficient preparation process (Yeo and Park, 2004), improving the final formulation performance, especially in drug delivery systems. It seems that the close contact between polymer and extract in the organic phase allowed that during the shell formation due to the polymer insolubilization, almost all the extract was entrapped into nanoparticle core.

One of the main problems related to the use of natural products is the patient acceptability. Children, pregnant, and elderlies reject drugs with unpleasant appearances, taste, and odor (Gibson, 2006). CgE has a pungent aroma and intense flavor. On the other hand, the pH, the presence of oxygen, the light, could affect the extract stability due to the presence of phenols, flavonoids, coumarins, and reducing sugars (Lafourcade et al., 2016). In this work, it was obtained a nanoparticle preparation of *C. grandis* extract, masking the unpleasant odor and flavor, and protecting the extract compounds from degrading agents like pH, light, and oxygen. In this form, CgND would be used without the stability and biopharmaceutical concerns related above.

The main factors affecting nanoparticle stability are the gravitational forces, particle aggregation and coalescence, and the Ostwald ripening (Tan et al., 2016). In nanoparticle systems, the drug-loaded content needs to remain intact along the time. The amount of the extract entrapped in nanoparticles kept between 83 and 84% without statistical difference along a year ($F = 1.88$; $p = 0.5321$, Table 1). Additionally, no changes were observed in nanodispersion, preserving the translucent aspect (Fig. 2) and practically odorless, suggesting the nanodispersion stability.

Conductivity is a crucial parameter for monitoring nanoparticles stability in suspensions (Roger et al., 2008). Factors like the nature of charges, temperature, and pH could affect the conductivity. The concentration and mobility of ions in solution governs the degree of conductivity. At the same time, pH expresses the H^+ and OH^- ion concentration in solution. The conductivity of the CgND remained less than 1.42 μS/cm along a year, with no statistically significant variation ($p > 0.05$, Table 1). At the same time, the pH remained stable (below 4.65) without statistically significant differences ($p > 0.05$, Table 1). At pH ≤ 5, Kollicoat®-coated nanoparticles remain strong and stable (BASF, 2010). It seems that CgND reaches an equilibrium, where counterions in solution interact harmonically with the nanoparticle electric double layer keeping the pH and conductivity practically unaltered.

The most common way for evaluating the stability of nanoparticle systems is monitoring the particle size, polydispersion index, and zeta potential along the time (Wu et al., 2011), in normal and stress conditions. In this work, these properties were evaluated every two months, along a year. The first 30 days, the particle size and polydispersion index diminished; onward this time remained almost constant. Along a year, no statistical difference was observed neither in particle size and polydispersion index nor zeta potential (Fig. 3). Tween 80 has an essential contribution to CgND stability. As a non-ionic surfactant, it is absorbed on the nanoparticle surface, producing a steric stabilization effect, avoiding aggregation and coalescence (Jamstorp et al., 2012). These results suggest that, after the first 30 days, CgND achieved a thermodynamic equilibrium, where the mains properties kept practically unaltered along a year (Fig. 3A–F), suggesting an excellent steric and electrical stabilization.

An adequate release profile is crucial for a good therapeutic effect. Among the main factors affecting the drug release are the pH, temperature, and the polymer nature. The release rate at pH 6.5 (SIF) was slower than a pH 7.4 (SIFwP) during the first

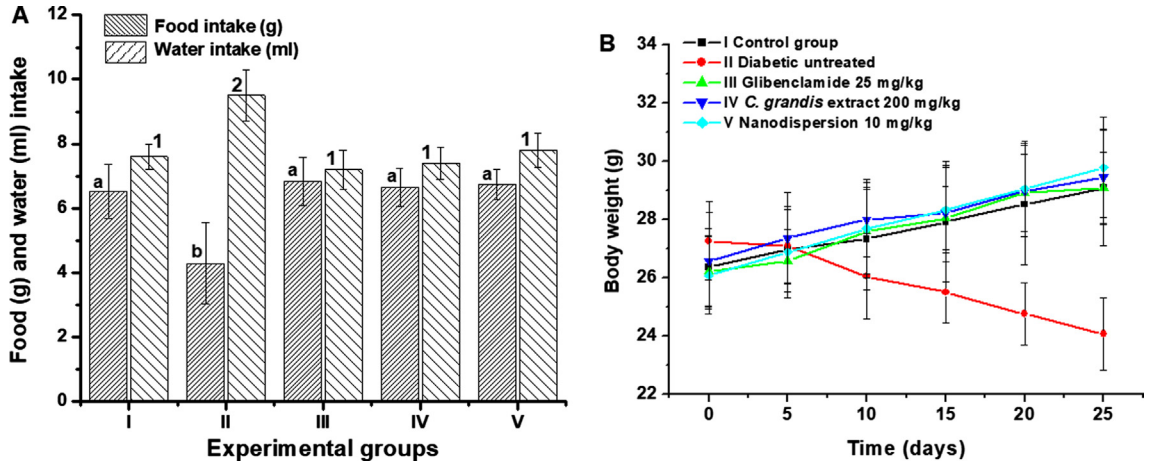

Fig. 5. Food and water intake (A) and the body weight (B) of the five experimental groups during the hypoglycemic activity evaluation. Different letter or number in a column indicates statistical differences.

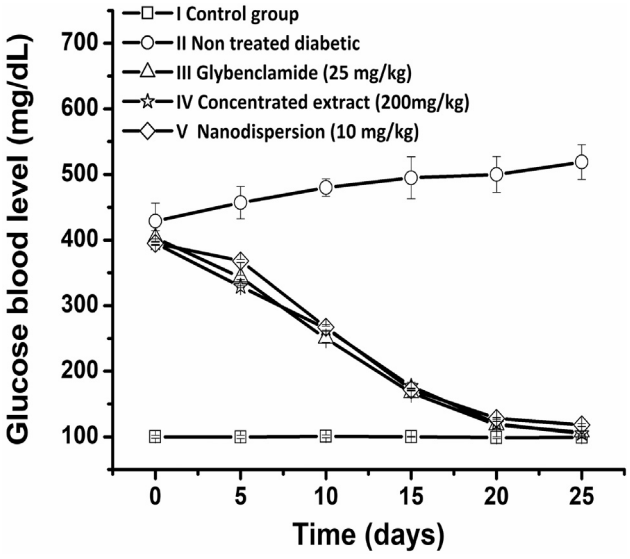

Fig. 6. Blood glucose levels of the experimental groups in the hypoglycemic activity assay.

60 min. Onward this time, the release rate in both mediums was statistically equal until release 100% at 120 min (Fig. 4). Kollicoat® MAE forms an acid-resistant coating layer at pH inferiors to 5.5; thus, nanoparticles do not dissolve at stomach pH (1.5–2.5). At pH above 5.5, the coating layer begins to swell and dissolve proportionally to the pH increase (BASF, 2010). In both mediums, the extract was slowly released from the nanoparticle core. This fact is essential for the therapeutic performance of this preparation. The acid-resistant property of the film formed by Kollicoat® MAE 100P and Tween 80, made feasible the use of CgND for intestinal release, protecting the extract from the stomach acid environment.

IC_{50} is the concentration of a substance required to inhibit 50% of an enzyme or a chemical radical. The IC_{50} of CgND in the DPPH assay was twice less than the IC_{50} of CgE and four times less than the gallic acid. In ABTS assay, the IC_{50} was twice less than the IC_{50} of CgE and five times less than the gallic acid (Table 2). CgND exhibited an antioxidant capacity far superior to the pure extract and the gallic acid (used as a control). Several studies reported a high correlation between the antioxidant capacity and phenolic content on natural extracts (Yashin et al., 2017). Thus, the presence of phenols, flavonoids, and coumarins in CgE and CgND could explain the potent antioxidant activity observed. Additionally,

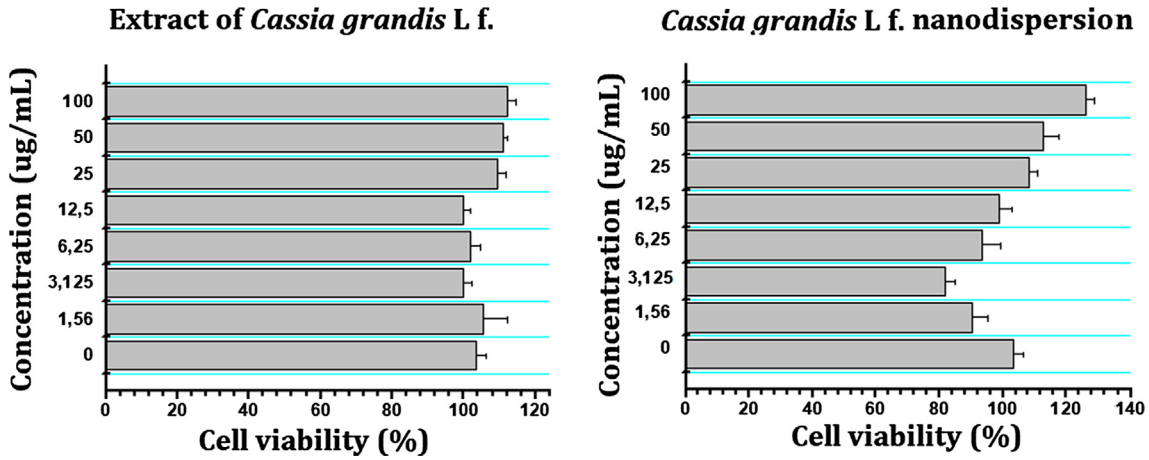

Fig. 7. Viabilities of MRC-5 cells exposed to *Cassia grandis* extract and *Cassia grandis* nanodispersion in different concentrations. The error bar represents the standard deviation of three replicates.

antioxidants compounds released slowly from nanoparticles could keep the reaction for longer, improving the antioxidant activity.

Pancreatic lipase is an enzyme involved in triacylglycerol absorption. The inhibition of this enzyme could be an effective way to alter the body fat absorption (Zhang et al., 2008). Studies have reported vegetal extracts lipase inhibitors, associating the activity with saponins, phenols, terpenes, glycosides, alkaloids, carotenoids and polysaccharides (Kim, 2007; Meenatchi et al., 2017). In this study, CgND exhibited an excellent lipase inhibitory effect with an IC_{50} value of 0.58 μg/mL, that was statistically different from the effect of Orlistat (standard lipase-inhibitor), which showed an IC_{50} of 0.29 μg/mL (Table 2). CgE (100 μg/mL) showed a low lipase inhibitory activity (4.1%). However, a nanodispersion prepared in the same way, but without CgE (blank), did not show lipase inhibitory activity, suggesting that the inhibitory activity observed in CgND was related, probably, with the slow release of the extract from nanoparticles and with the low particle size.

Lipase inhibitor drugs have potential application in the management of obese patients with type II diabetes (Kim, 2007; Meenatchi et al., 2017). There are no lipase inhibitors from plant extracts in the clinical use. However, apart from Orlistat, no new studies nor new molecules have been reported as lipase inhibitor (Lunagariya et al., 2014). The Orlistat® and others lipase-inhibitors produce gastrointestinal effects (i.e., fecal incontinence, flatulence, and steatorrhea (Birari and Bhutani, 2007). Considering this, CgND could be a promising product for assisting the obesity treatment in diabetic patients. Nonetheless, more studies have to be made to confirm the utility of this product in this health condition.

Alpha-glucosidase inhibitors (i.e., Acarbose, Voglibose, and Miglitol), are drugs helping to keep the blood glucose within the normal values. These drugs act by reducing the conversion rate of complex carbohydrates in their monosaccharides (Li et al., 2005). Alpha-glucosidase inhibitors are useful in Type II diabetes because the slow increase in blood glucose can be easily controlled. For this reason, substances with α-glucosidase inhibitor effect are excellent candidates for diabetes complementary treatment. CgND exhibited an excellent effect α-glucosidase inhibitor with an IC_{50} of 3.96 ± 0.5 μg/mL (Table 2). The effect of CgND was far superior to CgE (IC_{50} 32.30 μg/mL) and Acarbose, the reference drug (IC_{50} 62.30 ± 1.30 μg/mL). This effect is probably associated with the nanoparticle size (10^{-9} m), and the slow release of compounds from nanoparticles. On the other hand, it was reported that antioxidant compounds as phenolics, flavonoid, coumarins (Compound presents in CgE and CgND), could impede the carbohydrates bonding to α-glucosidase, producing a potent enzyme inhibition (Kusirisin et al., 2009). This result suggests that CgND could be a useful product for the diabetes management.

In diabetic patient increases the amount of free radical in blood by oxidative stress mechanisms (Scott and King, 2006). The progressive accumulation of reactive species on tissues and organs produces other diabetes complications like retinopathy, nephropathy, neuropathy, and macrovascular atherosclerosis, among others (Cruz et al., 2011; Gehl et al., 2016; Bonnefont, 2002; Scott and King, 2006). In this work, the hypoglycemic effect of CgND was evaluated in alloxan-induced diabetic mice. Alloxan destroys partial or totally, the pancreatic islets of Langerhans β-cells. This effect occurs mainly, by an oxidative mechanism (Chaudagar and Mehta, 2014, Mingueneau et al., 2015). In that condition, the insulin secretion decreases, producing high values of blood glucose, that are eliminated in the urine. At this point, triglycerides and cholesterol increase, producing obesity and other health problems (Bonnefont, 2002; Scott and King, 2006).

During the hypoglycemic experiment, animals of all groups consumed food and drank water normally (Fig. 5A). The intake of water in the Group II was superior and statistically different than the other groups, which were statistically, equal. At the same time, the ingestion of food in the Group II was minor and statistically different from the other groups, which were statistically equal. The low intake of food caused a body weight diminution on animals of the Group II (−11.44%, Fig. 5B). Probably, insufficient insulin secretion could cause a high loss of glucose and proteins in urine (Bonnefont, 2002; Scott and King, 2006), justifying the significant water intake. The control group showed normal blood glucose levels (Fig. 6), while in the negative control group (II) increased up to 450 mg/dL, due to non-treated diabetes. The hypoglycemic effect of CgE 200 mg/kg and CgND 10 mg/kg were statistically equal to Glibenclamide 25 mg/kg.

Besides the antioxidant effect, CgND also showed a potent α-glucosidase and lipase inhibitory activity, and both effects have significant utility in the type II diabetes management, for controlling the glucose absorption and diabetes complications (Mohamed et al., 2012). Probably, the hypoglycemic effect occurs via α-glucosidase inhibition. This fact could be associated with the pancreatic lipase inhibition and the slow release of the antioxidants from the nanoparticle core, acting synergistically to produce an effective control of type II diabetes.

The cytotoxicity *in vitro* of CgE and CgND against the non-neoplastic MRC-5 cells was evaluated (Fig. 7). The cellular viability in all concentrations of CgE was greater than 95%. In the same way, in all concentrations of CgND, the cellular viability was higher than 85%. According to these results, neither the CgE nor CgND possesses cytotoxicity *in vitro* in this cellular line. Some native populations have consumed the fruit pulp of Cassia grandis for centuries. Up to the best of our knowledge, this is not reports about any toxic occurrence caused by the fruit of this plant. This result suggests the innocuousness of this plant fruit, however, even though these substances were safe against MRC-5 cell line, tests in other cell lines should be performed.

5. Conclusions

Physicochemical properties of the CgND remain practically intact along a year stored in standard laboratory conditions. The slow release of the antioxidant compounds present in CgE from nanoparticle core could be contributing to the antioxidant effect and the lipase and alpha-glucosidase inhibitory activity. CgND in a dose 10 mg/kg exhibited a hypoglycemic effect statistically equal to glibenclamide (25 mg/kg). Cassia grandis extract and the nanodispersion are not cytotoxic against a non-neoplastic MRC-5 cell line; nonetheless, other tests should be performed in other cell lines, to warrant the safety use of this product. CgND is a promising nanotechnological product that could be used for the diabetes treatment. Additionally, the α-glucosidase and lipase-inhibitory effects of CgND could be useful in the obesity management. However, more studies have to be made to confirm the utility of this product in obesity.

Conflict of interest

The authors declare that there is no competing interest.

Acknowledgements

The authors appreciate the essential support of the CAPES/FAPEAP Cooperation program, Grant number 23038.000516/2013-01 and CAPES PNPD fellowship program.

ALP works in all stage of the experimental work. HK made the diabetes evaluation in experimental animals. LDRA and MJAS made the antioxidant, enzymatic and cytotoxic assays. JCTC, ESL, and TPS do the proofreading and contribute to the manuscript preparation.

JRRA design and coordinate the research process, translate, and the preparation of the final manuscript proofreading.

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