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In vivo schistosomicidal activity of (±)-licarin A-loaded poly (ε-caprolactone) nanoparticles

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ABSTRACT

Schistosomiasis mansoni is an infectious parasitic disease caused by worms of the genus Schistosoma, and praziquantel (PZQ) is the medication available for the treatment of schistosomiasis. However, the existence of resistant strains reinforces the need to develop new schistosomicidal drugs safely and effectively. Thus, the (±)-licarin A neolignan incorporated into poly-ε-caprolactone (PCL) nanoparticles and not incorporated were evaluated for their *in vivo* schistosomicidal activity. The (±)-licarin A -loaded poly(ε-caprolactone) nanoparticles and the pure (±)-licarin A showed a reduction in the number of worm eggs present in spleens of mice infected with *Schistosoma mansoni.* In addition, the (±)-licarin A incorporated in the concentration of 20 mg/kg and 200 mg/kg reduced the number of worms, presenting percentages of 56.3% and 41.7%, respectively.

1. Introduction

Schistosomiasis mansoni is a transmissible parasitic disease caused by worms of the genus Schistosoma, whose intermediate hosts are freshwater snails of the genus Bimphalaria, which can evolve from asymptomatic to extremely severe clinical forms. Praziquantel (PZQ) is the medication available for the treatment of schistosomiasis. Nevertheless, the existence of the possibility of resistant strains supports the need to develop new schistosomicidal drugs safely and effectively (Ismail et al., 1999; Sanchez et al., 2019). Thus, the interest of researchers in the isolation and identification of new natural compounds that can be used against various types of diseases is growing. Among them, neolignans and lignans stand out, which constitute a class of natural products with a significant variety of chemical structures and have the most diverse biological properties (Bastos et al., 1996; Macrae and Towers, 1984; Pereira et al., 2011a; Santos et al. 2019, 2021a; de Lima et al., 2021; Lima et al., 2021) such as dihydrobenzofuran (±)-licarin A (LIC) neolignane (Pereira et al., 2011a and Dias et al., 2013). The racemic mixture of LIC obtained by synthesis demonstrated potent trypanocidal and schistosomicidal activities (Pereira et al., 2011a). Thus, pure LIC neolignan and the LIC incorporated into poly-ε-caprolactone (PCL) nanoparticles were evaluated *in vivo* against Schistosoma mansoni. Poly-ε-caprolactone polymer (PCL) is a polyester widely utilized in drug delivery systems. It was chosen due to its elevated permeability, lack of toxicity, biocompatibility, and bioavailability, which is suitable for the controlled substances release of drugs. This work aimed to find a promising agent for the release of lignans and neolignans, opening perspectives for studies of new formulations that can increase the effectiveness of pharmacology action *in vivo* on *Schistosomiasis mansoni* of the neolignan studied here. In addition, the LIC

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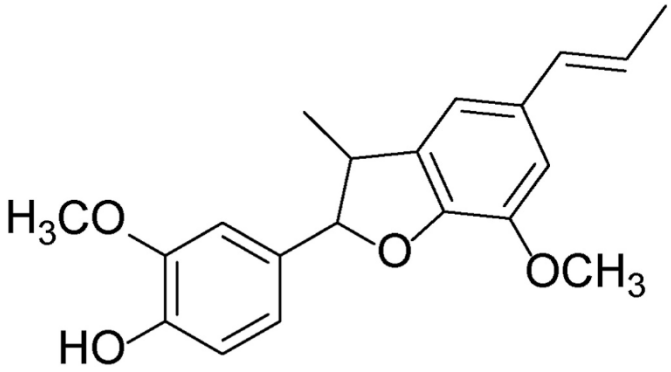

Fig. 1. Chemical structure of the (±)-licarin A.

incorporated into poly-ε-caprolactone (PCL) nanoparticles was chosen due to its properties of high permeability, lack of toxicity, biocompatibility, and bioavailability, which are suitable for the controlled release of drugs.

2. Material and methods

2.1. Equipment and materials

The Chromatography system consisted of a two-pump Shimadzu chromatograph model LC-20A Prominence equipped with a SIL-20A auto sampler, a column oven CTO-20A, a CBM-20A communications bus module, a DGU-20A3 in-line degasser, and an SPD-M20A photodiode array detector. The LC solution® software was used to process the data. The analyses were conducted on a LiChrospher RP-18 column (125 × 4.0 mm i.d., 5 μm). Acetonitrile (chromatographic grade) was supplied by Mallinkrodt Baker Inc. (Phillipsburg, NJ, USA). Water was cleaned with a Milli-Q-plus filter system (Millipore, Bedford, MA, USA). The NMR spectra were operated on a Bruker DPX 400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). The samples were dissolved in Dimetilsulfóxido (DMSO). The PZQ Pharmaceutical Secondary Standard was obtained from sigma Aldrich.

2.2. Obtainment of (±)-licarin-A

(±)-licarin-A (LIC) was obtained by the oxidative coupling reaction of isoeugenol (Fig. 1) (Pereira et al., 2011b).

2.3. Validation of the analytical method for quantification of (±)-licarin A by high performance liquid chromatography

The validation of the analytical method was performed according to criteria determined by the ICH (International Conference on Harmonization - ICH, 1996). The parameters investigated in the method validation were linearity, selectivity, accuracy, precision, recovery, quantification and detection limits, repeatability, and robustness. For the construction of the calibration curve, an external standardization was used from standard solutions. The Chromatographic analyses were performed through RP-HPLC-PDA using a LiChrospher RP-18 column (125 × 4.0 mm i.d., 5 μm), acetonitrile (solvent B), and water (solvent A) in gradient conditions as follows: 50–100% of B (0–15 min) as mobile phase, The temperature of the column was set at 30 °C, the flow rate was 1.0 mL/min, the injected volume was 20.0 μL, and the detection wavelength was set at 246 nm.

2.4. Preparation of standard solutions

A 1.00 mg mL^{-1} stock solution was prepared by dissolving 2.5 mg of LIC in 25.0 ml of acetonitrile (ACN). After diluting the stock solution, the working solution (20 μgmL-1) was obtained. Seven standard concentrations (1.00; 2.00; 4.00; 8.00; 12.0; 16.0 and 20.0 μgmL^{-1}) were prepared by diluting the working solution with exact amounts of ACN. All points on the calibration curve were prepared and analyzed in quintuplicate (n = 5). the calibration curve was constructed with the standard solutions; in addition to establishing the linear working range and determining the limits of quantification (LOQ) and detection (LOD) of the analytical method was calculated on the standard deviation of the response (σ) and of the slope of the analytical curve (S), using the expressions LOD = 3.3σ/S and LOQ = 10σ/S.

Table 1
Robustness of the chromatographic method.

Factors	Unit	Limits	Level (−1)	Level (+1)	Nominal
Flow rate	mL. min^{-1}	±0.2	0.98	1.02	1.0
Temperature	°C	±2,0	28	32	30
Mobile Phase Concentration	%	±2,0	48	52	50

2.5. Selectivity and linearity

Linearity was determined in triplicate by visually evaluating the external calibration curve and calculating the linear regression equation (A = ax ± b) and the correlation coefficient (r^2). In evaluating the selectivity, the chromatograms of prepared nanoparticle samples containing LIC were compared with nanoparticle samples without LIC (blank).

2.6. Accuracy and precision

By calculating the percentage of recovery of LIC, the Accuracy can be assessed, being [Accuracy = (average of the nominal concentration/concentration).100]. Three solutions at concentrations of 1.20, 10.0, and 18.0 μg mL^{-1} were carefully prepared. These concentrations were chosen because they cover the entire linear working range but at different concentrations from those used in the calibration curve. The determination of precision and Accuracy, each concentration was prepared in quintuplicate (n = 5). To confirm the validation of the analytical method, an analysis of variance (ANOVA) of the linear regression data was performed to assess the significance of the proposed method. Statistical significance was established at a P-value <0.05, which indicates that the proposed regression explains the model at a 95% confidence interval.

2.7. Robustness

Changes in the chromatographic method were analyzed by individual analysis according to the variations proposed in Table 1, thus verifying the method's ability to provide unaltered results when subjected to minor changes such as temperature, mobile phase concentration, and solvent flow, among other factors that can be changed. Robustness values were expressed as RSD (%) of the responses.

2.8. Preparation method of unloaded and loaded PCL nanoparticles containing LIC

Nanoparticle incorporations were performed using the nanoprecipitation technique described by Fessi et al., (1989). An organic phase was prepared, composed of a polymer (PCL 45), LIC, a low hydrophilic-lipophilic balance surfactant (Span® 60), and isodecyl oleate in acetone at 45 °C. This organic phase was slowly poured with a peristaltic pump, operated at a flow rate of 1.0 mL/min (10%) of its capacity, onto an aqueous phase containing Tween® 80 under moderate

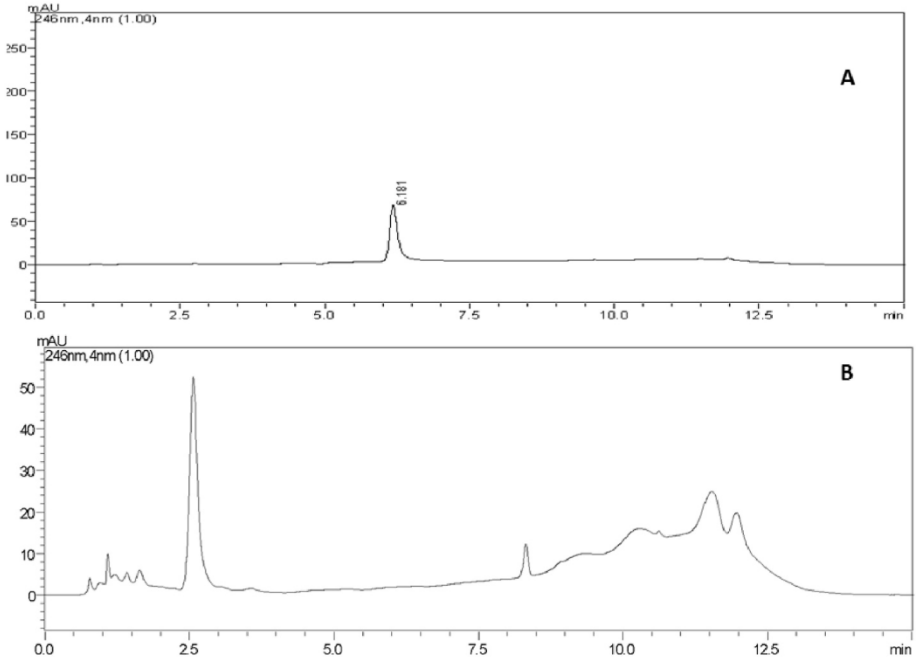

Fig. 2. Chromatogram of LIC (A) and chromatogram obtained from the nanoparticle without LIC (B).

magnetic stirring. Nanoparticles were formed immediately after diffusion of the organic solvent. The system was maintained under magnetic stirring for 10 min and at rest for 30 min. After this period, the organic solvent and part of the aqueous phase were removed under reduced pressure, adjusting the colloidal dispersion's final volume to a volume of 50 mL. The nanoparticles prepared for the *in vivo* assay followed the same method and reduced the final volume of the colloidal dispersion to 10 mL. The final materials were named PCL (control without LIC), LIC/PCL 200 (containing 200 mg of LIC), and LIC/PCL-20 (containing 20 mg of LIC).

2.9. Encapsulation efficiency (EE%)

Encapsulation efficiency (EE%) was assessed by quantifying the compound's free concentration in the colloidal suspension's dispersion medium (aqueous phase). A 0.5 mL volume of the colloidal suspension was filtered by centrifugation (958×*g*, 20 min) at 6 °C using Corning® tubes containing a cellulose acetate membrane with a 0.22-μm pore size that retained the nanoparticles while the free compounds were collected into the filtered solution. For HPLC analysis, a 0.3 mL volume of the filtered solution was evaporated and resuspended in 0.2 mL of acetonitrile. Encapsulation efficiency was calculated using equation 1:

$$EE\ (\%) = \frac{Total\ amount\ of\ compound\ -\ Free\ amount\ of\ compound}{Total\ amount\ of\ compound} x100$$

2.10. Physical-chemical analyses of the colloidal suspension

The physical-chemical analyses of the nanoparticles were conducted after the preparation of the colloidal suspensions. The parameter evaluated was the particle diameter (PD), assessed by the dynamic diffusion of the light analysis and the zeta potential (ζ). Measurements, analyzed in triplicate (n = 3), were obtained at room temperature at a fixed angle of 90°. In addition to the zeta potential in millivolts, this technique assessed the mean hydrodynamic diameter of the nanoparticles and the polydispersity index. Particle diameter and zeta potential were determined after dilution of 0.1 mL of each colloidal suspension in 10 mL ultrapure water and 10 mM NaCl (ZETASIZER 3000 HSa, Malvern Instruments).

2.11. In vitro release kinectics of the nanoparticles

The in vitro release studies of the nanoparticles in colloidal suspension were performed using the dialysis membrane diffusion technique proposed by Levy and Benita (1990). Dialysis tubing cellulose membranes, with 1 cm width, containing 1 mL of colloidal suspension, were tied up and placed in 1 L phosphate-buffered saline (PBS) with pH 7.4. This solution was kept under constant magnetic stirring. PBS was prepared as follows: 8.00 g NaCl; 0.20 g KCl; 1.44 g Na2HPO4·2H2O, and 0.24 g KH2PO4 for each liter of water. The system was kept at 36 °C with a 3 mL.min-1 steady flow controlled by a peristaltic pump. A dialysis membrane was removed from the solution at pre-determined intervals and analyzed by HPLC the LIC content.

2.12. Morphological analysis of the nanoparticles by scanning electron microscopy (SEM)

The nanoparticles were dried on an aluminum base covered with a thin layer of gold. Morphology and homogeneity of the nanoparticles were evaluated under scanning electron microscopy using a TESCAN VEGA 3 SBH apparatus equipped with a 30-kV W filament.

2.13. In vivo evaluation of schistosomicidal activity

2.13.1. Parasite strain

S. mansoni LE (Luiz Evangelista) strain was maintained through *Biomphalaria glabrata* snails and BALB/c mice (Pereira et al., 2015). The University approved all Franca Animal Research Ethics Committee procedures under protocol #005/09-A.

2.13.2. Experimental infection and treatment of laboratory animals

BALB/c female mice (20–30 g body weight; n = 7 animals per group) with six-week-old were experimentally infected with 80 cercariae using a method where the animal's tail is exposed to a medium containing the cercariae, as described by (Radke et al., 1971). Forty-nine days post-infection, pure LIC and PZQ compounds were dissolved in 200 μL of saline: EtOH: DMSO (50:25:25) and administered as a single dose by gavage according to the treatment group; LICPCL 20 and LICPCL200 formulations, containing 20 mg/kg and 200 mg/kg of incorporated LIC,

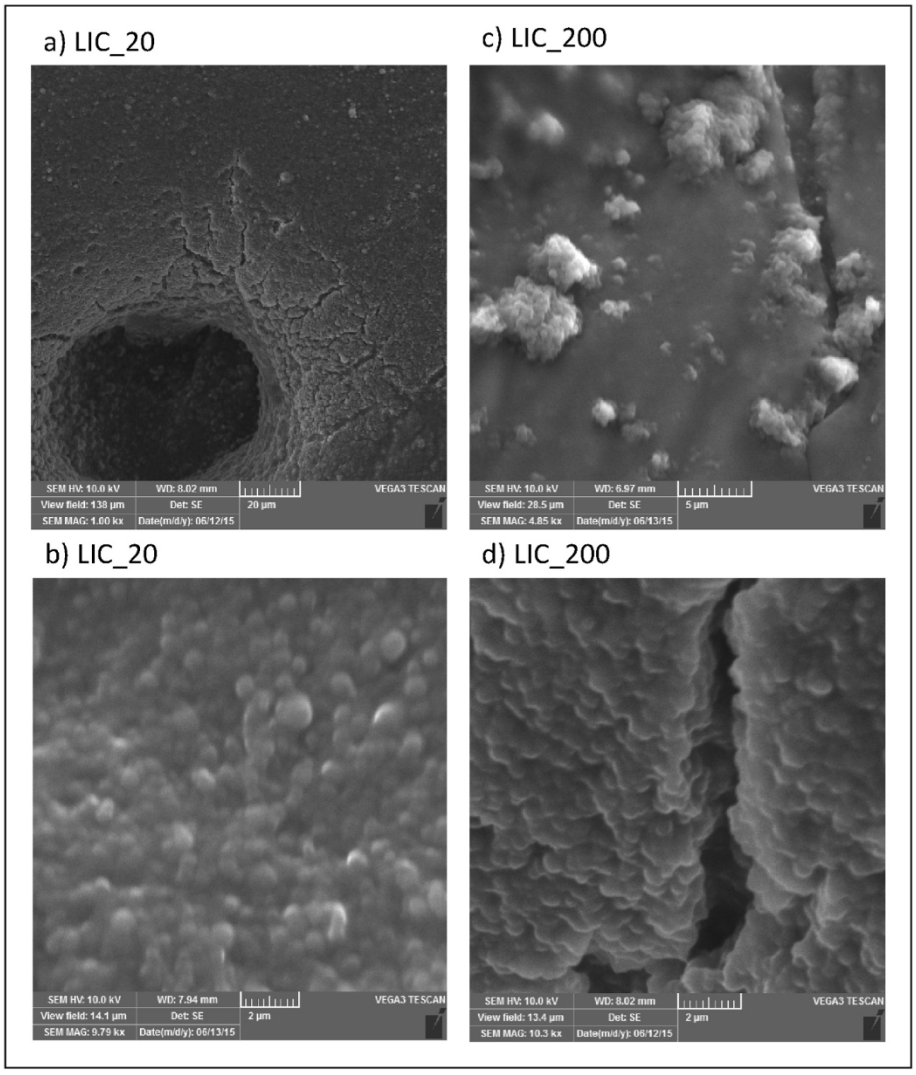

Fig. 3. SEM image of PLC particles containing embedded LIC at different concentrations. LIC_20 (20 mg/kg of (±)-licarin-A) and LIC_200 (200 mg/kg of (±)-licarin-A). It can be seen in nanoparticles in evidence (a and c) and agglomerated (b and d) that presented differences in structural organization due to different concentrations of the active compound.

respectively, were administered in suspension. As a negative control group, mice were treated with 200 μL of saline: EtOH: DMSO; 50:25:25 or PCL without LIC, while the positive control group received 400 mg/kg PZQ (Yepes et al., 2014). The liver and the spleen were collected and weighed. A small fragment of the anterior medial lobe from each liver was digested in a solution of 4% KOH at 37 °C, under agitation, for 12 h (Cheever, 1970). The solution obtained after digestion was centrifuged (15,280×*g*, 26 °C) (Centrifuge 5417R- Eppendorf) under agitation (5200×*g*, 10 min), and the resulting sediment was placed on a plate to count the number of eggs under microscopy (Primo Vert Microscope). The reduction of egg numbers in the liver and spleen was calculated as previously described for adult worms.

2.13.3. Histopathological analysis

A liver fragment from each mouse was collected and kept in PBS containing formalin 10%. This material was taken to the Histology Laboratory at the University of Franca, where 5-mm thick histology slices were obtained and stained with Masson's trichrome to analyze the granuloma area under light microscopy (Primo Vert microscope). For that, images were captured using an AxioCamERC5S camera connected to the microscope with 4X and 10× objective lenses and analyzed using the AxioVision software (Carl Zeiss). Thirty granulomas from each animal were evaluated, and the granuloma areas were calculated as the mean granuloma area for each treatment group.

2.13.4. Statistical analysis

Results are expressed as mean ± standard deviation. The one-way Analysis of Variance (ANOVA) and Dunnett's test were used to compare results between the treated and the control groups. Statistical analyses were executed utilizing the GraphPad Prism software (Version 5.0 for Windows, GraphPad Software, San Diego, California, EUA).

3. Results and discussion

3.1. Development and validation of the analytical method

For qualitative and quantitative control of LIC in nanoparticles, it was necessary to develop an analytical method by HPLC. The synthesized compound showed a high degree of purity. The elution time of the compound was detected at 6.2 min (Fig. 2). The wavelength showed the maximum absorption in the region of 246 nm. The developed method presented linearity in 01.0–20.0 μg mL^{-1}. After adjusting the data by linear regression using the least-squares method, the values of the areas obtained were shown to be directly proportional to the concentration of

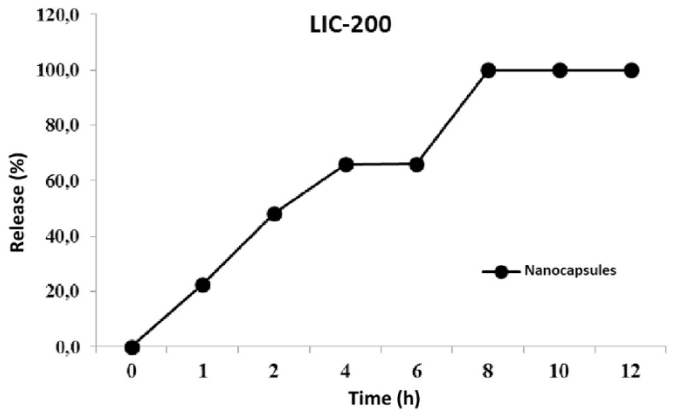

Fig. 4. *In vitro* release kinetics of polymeric nanocapsules loaded with 200 mg/kg of (±)-Licarin A (LIC_200).

LIC with equation y = 2733.104 x – 2717 and $r^2 = 0.9932$. The limit of quantification (LOQ) corresponded to 1.00 μg mL^{-1}, and the limit of detection (LOD) was 1.35 μg mL^{-1}. The results obtained by the HPLC analysis of LIC solutions in ACN with the presence of components for the preparation of nanoparticles (PCL, tween® 80, and span® 60). The analysis of the LIC neolignan-free nanoparticles by HPLC showed that this polymer did not affect the drug quantification, being the method selective (Fig. 2). The relative standards deviation (RSD%) to intra-day and inter-day precision was lower than 2.0%. The results showed that the method is precise for determinations of LIC. The method's accuracy was also evaluated through quintuplicate analysis of low, medium, and high concentrations, showing that the method developed for LIC is accurate since the deviations from the nominal values remained between 91.17 and 113.7% for the same day of analysis and analysis on different days. Values are by the criteria established for the method, with variability less than or equal to 20% (Santos et al., 2021b; Silva et al., 2021). The method's robustness was analyzed by examining the effects of changes on the rrt (relative retention time) and concentration to calculate the effects (Ex) that were converted to RSD%. Finally, the analysis of RSD% showed that all factors influence the method equally, with a value of less than 22% considering all the factors analyzed.

3.2. Morphological analysis and characterization of nanoparticles formulations

PCL nanoparticles containing the LIC compound were characterized by scanning electron microscopy (SEM) for morphology evaluation. It is possible to observe, according to Fig. 3, a photomicroscopy of nanocapsules containing LIC.

The average size of nanocapsules was approximately 18 nm in evidence and agglomerated, presenting a difference in structural organization due to different concentrations of the active compound. The nanoprecipitation method incorporates (±)-licarin A into poly (ε-caprolactone). The formulations containing the LIC compound presented a homogeneous and opaque aspect due to the particle diameter, 233.2 for LIC_220 (200 mg/kg) and 131.4 nm for LIC_20 (20 mg/kg), in addition to both presenting ease in the incorporation process.

Already LIC_200 precipitate after a few hours of the incorporation process due to the high concentration of the active compound, thus affecting the nanoparticle's structure stability. Both nanoparticles had a negative ZP of −39.9 mv for LIC_220 and LIC_20. Nanoparticles with presented ZP value can positively influence the release kinetics, as it has good colloidal stability in solution (Lima et al., 2021).

The recovery percentage obtained by the HPLC analysis was 39.1% for LIC_200, while the formulation with LIC_20 showed a 61% recovery. The results analyzed are as expected. The in vitro release kinetic profiles of polymeric nanoparticles loaded with LIC_200 is shown in Fig. 4. The system designed under constant flow avoids system saturation and, consequently, solubility equilibrium (Lima et al., 2021). LIC showed a rapid release at the beginning, reaching 40% release in 4 hands between 6 and 8 h. There was a release of around 60%. After the 8 h of analysis, the release was 100% up to the final 12 h. Thus, presenting an excellent initial release that can result in a better biological effect, as the release kinetics is directly related to the concentration of the compound, which is one of the factors that change the bioavailability of a drug.

PCL is a polyester widely used in the application of drug delivery systems. Furthermore, it is much more resistant to chemical hydrolysis, which may limit the possibility of property modulation of the system delivery through the structural configuration of the polymer chains. It is a crystalline, highly hydrophobic polymer that slowly degrades without enzymes. The properties of the polymer and its behavior with the environment (pH in body fluids) determine the drug release rate. Some studies showed that in alkaline conditions, it has a pH-responsive drug release effect (Sudhakar et al., 2014). in addition, LIC_200 was the dosage that allowed better-sustained drug release.

3.3. Effect of (±)-licarin-A nanocapsules in animals infected with S. mansoni

The previous study demonstrated that the racemic mixture of LIC had significant schistosomicidal activity in vitro with a CC50 value of 53.57 μM (Pereira et al., 2011a). An acute toxicity study showed that LIC is toxic at doses greater than 300 mg/kg (Souza et al., 2013). Therefore, to carry out the present work experiments, concentrations below 300 mg/kg were determined, being 200 mg/kg for evaluation of the pure substance, and when incorporated into nanoparticles, the

Table 2
Results of the *in vivo* experiment after treatment with praziquantel, pure (±)-licarin-A and nanocapsules (±)-licarin-A (LIC/PCL).

Evaluated aspects	Diluent Control 1[a]	Diluent Control 2[a]	PZQ 400 mg/Kg	LIC 200 mg/Kg	LIC/PCL 200 mg/Kg	LIC/PCL 20 mg/Kg
Mean blood worms ± S.E.M	20.40 ± 4.98	24.20 ± 7.92	3.20 ± 2.77***	11.38 ± 5.21	14.11 ± 7.50*	10.58 ± 5.93*
Total reduced worms[b] %	–	–	85.0	44.2	41.7	56.3
Liver Weight (g)	1.85 ± 0.36	1.67 ± 0.27	1.70 ± 0.13	1.60 ± 0.13	1.23 ± 0,20**	1.33 ± 0.10*
Spleen Weight (g)	0.40 ± 0.08	0.31 ± 0.05	0.30 ± 0.01	0.28 ± 0.05*	0.24 ± 0.06	0.19 ± 0.04*
N° eggs/g in liver	5494 ± 1086	5953 ± 1152	4164 ± 1341	3195 ± 1037	4457 ± 284.7	4340 ± 1134
reduced eggs[b] % in liver	–	–	24.21	42.00	25.21	27.10
N° eggs/g in Spleen	5070 ± 1077	903.0 ± 763.4	37.4 ± 44.15	17.0 ± 13.11***	316.2 ± 328.5**	46.20 ± 55.26***
reduced eggs[b] % in Spleen	–	–	92.7	96.6	65.0	95.0

The asterisk indicates statistically significant differences compared to the control group diluent 1 (Saline:EtOH:DMSO 50:25:25) and diluent 2 (poly-ε-caprolactone) (*P < 0.05; **P < 0.001; ***P < 0.0001).

[a] Statistical of significant difference compared to negative control (Diluent 1 and 2).

[b] Percentages of reduction are calculated according to the equation: % = [(value of untreated control group – value of treated group)/value of untreated control group] X 100%. Results are shown as mean ± Standard Deviation.

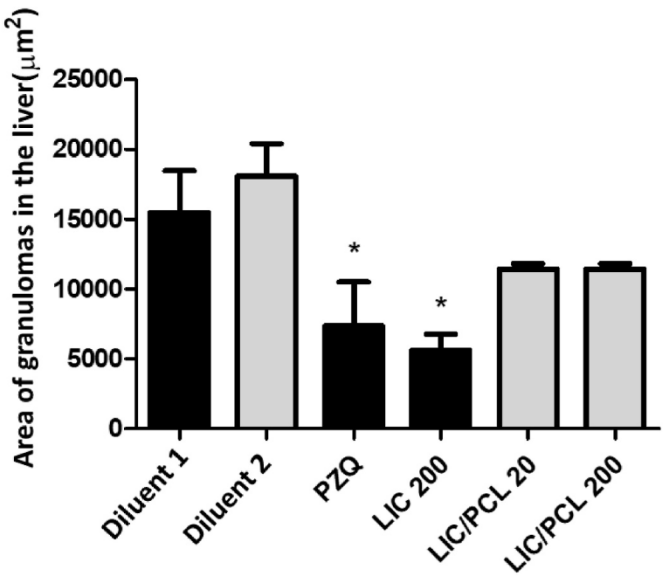

Fig. 5. *In vivo* effect of (±)-licarin-A in reducing the area of granulomas in the liver. (*P < 0.05).

concentrations were 200 and 20 mg/kg. Although the LIC incorporated in the concentration of 20 mg/kg and 200 mg/kg significantly reduced the number of worms compared to the diluent 2 (poly-ϵ-caprolactone), presenting percentages of reduction worms 56.3% and 41.7% (Table 2). That it can be considered with low schistosomicidal activity when compared to PQZ. The same behavior is observed for the LIC pure at 200 mg/kg (Table 2). During the liver weight (g) evaluation, there was more significance for the groups of LIC incorporated at concentrations of 20 and 200 mg/kg than the pure substances evaluated. The treatment with pure LIC at a 200 mg/kg concentration was reduced by 42%. The egg number in the liver is a better result than the PZQ, which was reduced by 24.21% (Table 2). When evaluated with the incorporated LIC (20 and 200 mg/kg), the reduction in the number of eggs was 27% and 25%, respectively (Table 2). Another organ evaluated was the spleen, verifying changes in weight and number of eggs. It was observed that the treatments with pure LIC (200 mg/kg) and incorporated LIC (20 mg/kg) significantly reduced the spleen weight (g) when compared to diluent 1 and diluent 2, respectively. Compared to the Diluent control groups, the number of eggs in the spleen showed significant results for all evaluated LIC groups. Thus, the incorporated LIC (200 and 20 mg/kg) and the pure LIC showed 65 and 96.6% egg reduction, respectively (Table 2). In addition, a 95% reduction was also observed when treated with LIC incorporated at a concentration of 20 mg/kg. These results evidence the low of pure LIC and incorporated LIC to reduce the total number of worms and number of eggs in the liver and demonstrated the significant activity, dose-independent of incorporated LIC in the reduction of eggs in the spleen, probably due to saturation of the receptors that would be responsible for the activity. Thereby, more additional studies are needed to elucidate this displayed behavior.

3.4. In vivo evaluation of histopathological aspects during experimental schistosomiasis

Histopathological analysis of animals infected with S. mansoni was evaluated by measuring the area of hepatic granulomas after treatments. When evaluating the treatment with LIC, there was a reduction in hepatic granulomas at 200 mg/kg doses compared to diluent 1 (Fig. 5). Encapsulated LIC did not show significant results. PZQ also showed a reduction in granulomas compared to diluents 1 and 2 (Figs. 5 and 6). Reducing the number of eggs in the liver is extremely important, as the egg is responsible for the significant pathogenesis of the severe phase of schistosomiasis (Gryseels et al., 2006). The decrease in the size of the granulomas may be related to a smaller number of eggs reaching the liver; in addition, the treatment may induce the regulation of the immune response, allowing the formation of granulomas but less aggressively (Gryseels et al., 2006). Treatment against schistosomiasis is based on the use of PZQ. However, concerns about the parasite's resistance to therapy and its widespread use have encouraged further research into new drugs (Gryseels et al., 2006; Pereira et al., 2015).

The mechanism by which LIC exerts the schistosomicidal effect *in vivo* is unclear. Some factors, such as the modulation of the redox cycle, and the induction of oxidative stress, in adult S. mansoni worms, must be

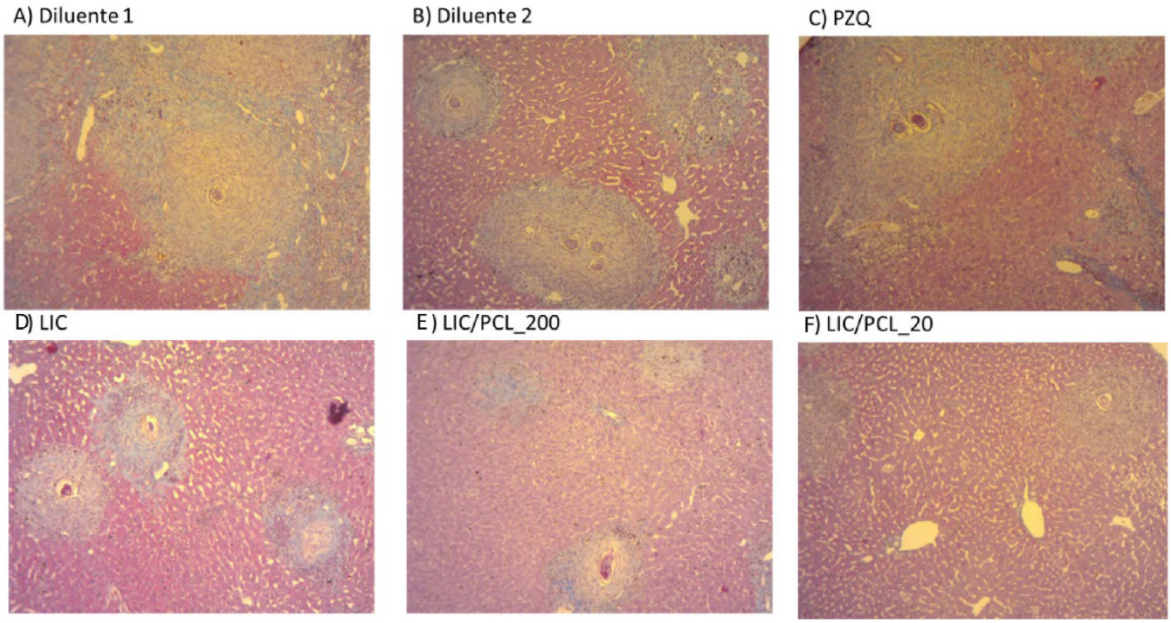

Fig. 6. Micrographs of hepatic granulomas from mice infected with *S. mansoni* after treatments. Panel A) Diluent 1; B) Diluent 2; C) Praziquantel; D) (±)-licarin-A 200 mg/kg; E) PCL incorporated with 200 mg/kg of (±)-licarin-A and F) PCL with 20 mg/kg of (±)-licarin-A. Images captured by an AxioCamERC5S camera attached to a microscope in a 10× objective.

considered to assess the drug's action. Previous studies indicated that the integument could be a target for developing schistosomicidal drugs. However, most of the compounds used against Schistosoma, such as PZQ and mefloquine, can act by damaging the integument and showing other mechanisms of action. LIC, a promising neolignan, has been studied by our research group and has potent trypanosomicidal and schistosomicidal activity in vitro and presents anti-inflammatory, antimicrobial, and antitumor activity (Pereira et al., 2011a; León-Díaz et al., 2010 and León-Díaz et al., 2013). This neolignan has some preferential pathways of metabolism in mice, for example, oxidation, demethylation, and dehydrogenation (Li & Yang, 2011). Investigations have been realized to understand its mechanism of action, thus proving the effectiveness of this promising substance.

4. Conclusion

The administration of LIC and LIC/PCL orally was viable since they do not show cytotoxicity effects. Instead, the pure LIC showed better results, demonstrating moderate schistosomicidal activity and confirming the previously described findings (Pereira et al., 2011a; León-Díaz et al., 2010; León-Díaz et al., 2013); thus, this is the first report on the *in vivo* schistosomicidal activity of (±)-licarin A. In addition, the nanoparticles were stable in aqueous dispersion. They can be optimized to be used as a promising agent for releasing bioactive compounds, opening perspectives for studies of new formulations that can increase the effectiveness of drugs and investigations on the mechanism of action of drugs with schistosomicidal activity.

Author statement

The authors have no competing interests to declare.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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