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Article

Improved antimutagenic effect of *Pyrostegia venusta* (Ker Gawl.) Miers nanostructured extract in liposome and polymeric nanoparticle

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Pyrostegia venusta (Ker Gawl.) Miers, popularly known as "*Cipó-de São-João*", has been used in traditional medicine for its therapeutic properties. Nanotechnology is able to enhance the pharmacological activity of plant extracts. In this context, liposomes and polymeric nanoparticles containing *P. venusta* ethanolic extract were developed and then physico-chemically characterized to evaluate the mutagenic/antimutagenic effects of *P. venusta*. In addition, transaminases and serum creatinine were biochemically analyzed for liver and renal damage, respectively. The micronucleus test was performed with male Swiss mice treated orally for 15 consecutive days with free extracts and nanostructured with *P. venusta*, and then intraperitoneally with N-ethyl-N-nitrosurea (50 mg/kg) on the 15th day of treatment. Micronucleated polychromatic erythrocytes (MNPCE) were evaluated in bone marrow. There was a significant reduction in the frequency of MNPCE (LP_{EPV} = 183% and NP_{EPV} = 114%, $p < 0.001$), indicating antimutagenic potential of the nanostructured extracts with *P. venusta*. The groups treated with only nanostructured extract did not show an increase in MNPCE frequency, and biochemical analyzes showed no significant difference between treatments. The liposomes and polymeric nanoparticles containing *Pyrostegia venusta* ethanolic extract showed biological potential in preventing the first step of carcinogenesis under the experimental conditions.

Keywords: *Pyrostegia venusta*. Flavonoids. Nanotechnology. Micronucleus test.

INTRODUCTION

Several toxic compounds have affinity with the organism and high capacity to react with our genetic material. When cells are exposed to a potentially toxic chemical, it is possible to then find small intracytoplasmic masses in the cell chromatin. These masses are found as a small cell nucleus in the cytoplasm outside the bigger nucleus, denominated the micronucleus (Queiroz *et al*., 2013).

The formation of this micronucleus may be spontaneous, however some compounds have the ability to intensify the incidence of lesions, and it can characterize a mutagenic event for cells and possible apoptosis avoidance (Kirkland *et al*., 2005). Therefore, chemoprevention is based on the use of natural or synthetic agents to reverse, prevent or suppress the carcinogenic progression

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(Oliveira, Aldrighi, Rinaldi, 2006). Thus, compounds with chemopreventive activity which occur in nature and can be obtained easily are of great relevance for public health and an alternative for reducing neoplasia rates (Huang, Plass, Gerhauser, 2011).

In this context, identifying chemopreventive agents is relevant as a possible strategy for cancer prevention. Some plants have demonstrated important chemopreventive and antineoplastic effects, but their use still has to be very careful, since many of them also present side effects (Kirkland *et al*., 2005; Queiroz *et al*., 2013).

Therefore, developing studies which enable better understanding about the use of these agents and their possible pharmacological activity, either physiologically or molecularly, can elucidate a better risk/benefit analysis and selection against diseases related to genotoxic events (Kirkland *et al*., 2005; Queiroz *et al*., 2013).

Pyrostegia venusta (Ker Gawl.) Miers belongs to the Bignoneaceae family, also known as *Pyrostegia ígnea*, or by its popular names of *Cipó* or *Flor de São-João*, and presents itself as an important natural source of antioxidants since it has a significant amount of secondary metabolites such as phenolic compounds which can behave as free radical inhibitors or suppressors (Roy *et al*., 2011; Altoé *et al*., 2014; Pereira *et al*., 2014). Studies show that leaf and flower ethanolic extracts are used in traditional medicine to treat white spots on the body, known as leucoderma or vitiligo (Magalhães *et al*., 2010).

Thus, nanotechnology is linked to the use of medicinal plants with relevant antioxidant activity, and is a science which develops technological products in nanometric scale. This connection aims to optimize therapeutic doses of vegetable compounds in stable physico-chemical structure with biological affinity. An increase in the therapeutic index, stability improvement, protection against physical and chemical degradation and maintenance of serum levels of nanostructured compounds have also been observed (Ourique *et al*., 2014). Plant extract matrices are complex and their tissue solubility and compatibility may be difficult. Thus, administration of these nanosystems is an effective alternative to reverse these factors (Lima, Albuquerque, 2012).

Among these drug nanocarriers, liposomes and polymeric nanoparticles are important types of carriers used in research. Liposomes provide a broad range of size varying from nanometers to micrometers. They are constituted by one or more concentric phospholipidic bilayers with dispersed amphiphilic characteristics (Ajazuddin, 2010; Pereira *et al*., 2014). Polymeric nanoparticles are mostly constituted by biodegradable and biocompatible synthetic polymers such as poly (ε-caprolactone) (PCL). These nanosystems containing bioactive compounds have been shown to have advantages because they act in disease/illness prevention or health promotion, promoting higher quality and safety for their use (Bitencourt *et al*., 2016).

In this context, the current study identified the presence of flavonoids in *P. venusta* leaf extract, evaluated the chemopreventive effect of the extract nanocarried in liposomes and polymeric nanoparticles on the damages induced to the DNA *in vivo*, as well as verified the possible hepatotoxic and nephrotoxic effects through hepatic transaminases (AST and ALT) and creatinine (CR) dosing, respectively.

MATERIAL AND METHODS

Animals

This research study was approved by the Ethics Committee on Animal Use (*CEUA* – UFMT/Cuiabá Campus, MT) (Protocol number: 23108.720740/2016-39), which is affiliated to the Council for Control of Animal Experiments (*CONCEA*). Male Swiss mice aged 6-7 weeks (weighing 25-30 g) were obtained from the breeding colonies of Central Biological Unit of the UFMT, Cuiabá campus. The mice were kept in plastic cages in an experimental room of LiPeQ/UFMT/Sinop-MT during the experimental period, under controlled temperature (22 ± 2 ºC), relative humidity (55 ± 10 %), light cycle (12 hours light/dark), exhaust system conditions and fed with pelleted feed diet and filtered water ad libitum.

Chemicals

N-ethyl-N-nitrosourea (ENU, Sigma Aldrich, Saint Louis, USA), polysorbate 80 (Delaware, Porto

Alegre, Brazil), poly (ε-caprolactone) with molecular weight (Mn) = 80.000 g/mol (PCL), sorbitan monooleate (Sigma-Aldrich, Lesquin, France), lipoid S75® (Sanbio Científica, São Paulo, Brazil) and caprylic/capric triglyceride (Brasquim, São Paulo, Brazil) were used in the experiment.

Pyrostegia venusta ethanolic extract

Pyrostegia venusta was collected in Sinop/MT at 11º52'12.8"S/55°31'18.4"W geographical coordinates in November of 2014, identified and stored at Herbário Centro-Norte Mato-Grossense (Voucher specimen CNMT 6207), UFMT/Sinop. The *P. venusta* extract was obtained from its leaves by drying and grinding them until obtaining a final mass of 2.39 kg. This material was macerated in 21.5 L of ethanol for 7 days; afterwards, the obtained extract was filtered and concentrated by rotary evaporator. The leaf ethanolic extract exhibited a yield of 5.6% with a mass of 135.7 g.

The flavonoids in the *P. venusta* leaf ethanolic extract were identified according to Duan *et al*. (2011) with modifications. First, a comparison between the retention time of the sample being analyzed and the authentic external standards prepared in methanol was performed to characterize these flavonoids. The standards used were: amentoflavone, apigenin, kaempferol, luteolin, myricetin, quercetin, quercetin-3-β-d-glycoside, rutin and taxifolin (Sigma-Aldrich). The analysis was conducted by high-performance liquid chromatography/Tandem mass spectrometry (LC-MS/MS) by UPLC Agilent 1290 Infinity (Agilent Technologies, USA), Agilent Eclipse AAA column (4.6 x 150 mm, 3.5µm) at 25 ºC, and 20 µL of injection volume. Mobile phase gradient with acidified water by formic acid 0.1% (m/v) and acetonitrile in 05:95 to 95:05 (ACN: H_2O) conditions. The run time was 33 min with a flow of 0.5 mL/min. Detection was obtained through mass spectrometry by Agilent 6460 Triple Quad with electrospray as ionization source and using nitrogen gas in the following conditions: gas temperature 300 ºC; flow 5 L/min; nebulizer pressure 45 psi; sheath gas temperature 250 ºC; flow 11 L/min; capillary - 3500 V and m/z scanning interval 120-900 units.

Preparation of nanostructures

The liposomes were developed by the reverse phase evaporation method (Mertins *et al*., 2005). An aqueous phase with 1 mg/mL *P. venusta* ethanolic extract (m/v) and 0.25% (m/v) polysorbate 80 was prepared in phosphate buffered saline pH 7.4 and an organic phase with soybean phosphatidilcholine (1.2 g) and cholesterol (0.06 g) in 40 mL of chloroform. An aliquot of the aqueous dispersion (4 mL) was added over the organic phase and sonicated for 5 min, obtaining inverted micelle dispersion. The organic solvent was removed at 25 ºC by evaporation under reduced pressure, resulting in an organogel. Next, the remaining aqueous solution (96 mL) was added to the organogel and the system was kept under stirring for 30 min. Vesicles suffered extrusion through 0.22 µm membrane. In addition to the liposomes containing *P. venusta* ethanolic extract (LP_{EPV}), liposomes without active extract (LP_{Bl}) were also developed, as described above.

Polymeric nanoparticles were developed by the solvent emulsification/evaporation method described by Quintanar-Guerrero *et al*. (1998) and Bitencourt *et al*. (2016). First, 1 mg/mL (m/v) of *Pyrostegia venusta* leaf ethanolic extract was dissolved in the aqueous phase containing 1% (m/v) polysorbate 80. The organic phase (ethyl acetate) was prepared with 1% (m/v) PCL and 1% (m/v) sorbitan monooleate. Both phases were subjected to gentle stirring at 40 ºC individually. The aqueous phase was then added over the organic phase after 60 min, originating the primary emulsion. This emulsion was kept under vigorous magnetic stirring for 20 min and a second aqueous phase containing 2% (m/v) polysorbate 80 was subsequently added over the first emulsion and transferred to a high shear mixer (mini homogenizer, TECNAL, model TE-103) at 6000 rpm for 10 min. Lastly, a rotary evaporator eliminated the solvent, originating the formulation called NP_{EPV}. Nanoparticles without the active extract (NP_{Bl}) were also prepared for comparison purposes, as described above.

All formulations were prepared in triplicate and kept under refrigeration at 8 °C protected from light.

Physicochemical characterization of the nanostructured formulations

The nanostructured formulations were macroscopically characterized according to their homogeneity, color and appearance. Formulations were microscopically evaluated according to their equivalent sphere average diameter (d4.3) and particle size distribution (Span) by laser diffraction (Malvern Mastersizer® 3000), confirmed through determining the average cumulative diameter (Z-average) and polydispersity index (PDI) by photon correlation spectroscopy analysis (Nanosizer® Nanoseries, Malvern Instruments, United Kingdom) at 25 °C. The pH determination was conducted by pHmeter (Gehaka, PGH2000) previously calibrated with pH 4.0 and 7.0 standards by directly inserting them into the formulations. Zeta potential (ξ) was determined through sample electrophoretic mobility (Zetasizer® Nano-ZS model ZEN 3600, Malvern Instruments, United Kingdom). Trials were performed in triplicate and the results plotted as the average of three determinations.

Micronucleus test in vivo

The acquisition and preparation of the erythrocyte slides from the bone marrow for the micronucleus (MN) frequency evaluation followed the methodology by MacGregor *et al*. (1987). A total of 1000 cells per animal were analyzed by light microscope, 1000 times magnification (immersion) for slides prepared and decoded in a blind test and duplicated for each animal.

The MN frequency reduction percentage was calculated according to Sugui *et al*. (2003), through the equation below:

$$\% \text{ reduction} = \frac{(\text{Frequency of MN in A}) - (\text{Frequency of MN in B})}{(\text{Frequency of MN in A}) - (\text{Frequency of MN in C})} \times 100$$

Where A represents the group treated with ENU (positive control), B represents the group treated with *P. venusta* plus ENU, and C represents the group treated with 0.9% NaCl (negative control).

Experimental design

The mice were divided into 9 groups of 6 animals each, as referred below:

Group 1 - Negative control. The mice were treated with LP_{Bl} via gavage with administration of 0.3 mL during all of the trial period. The mice were intraperitoneally treated with 0.9% NaCl (0.1 mL/10g p.c.) on the 15th day; the mice were sacrificed on the next day (16th day) by decapitation followed by collecting cells from the bone marrow.
Group 2 - Negative control. The mice were treated with NP_{Bl} via gavage with administration of 0.3 mL during all of the trial period. The mice were intraperitoneally treated with 0.9% NaCl (0.1 mL/10g p.c.) on the 15th day; the mice were sacrificed on the next day (16th day) by decapitation followed by collecting cells from the bone marrow.
Group 3 - Positive control. The mice were treated with LP_{Bl} via gavage with administration of 0.3 mL during all of the trial period. The mice were intraperitoneally treated with ENU (50 mg/kg p.c.) on the 15th day; the mice were sacrificed on the next day (16th day) by decapitation followed by collecting cells from the bone marrow.
Group 4 - Positive control. The mice were treated with NP_{Bl} via gavage with administration of 0.3 mL during all of the trial period. The mice were intraperitoneally treated with ENU (50 mg/kg p.c.) on the 15th day; the mice were sacrificed on the next day (16th day) by decapitation followed by collecting cells from the bone marrow.
Group 5 - Group treated with LP_{EPV} (1 mg/mL) via gavage with administration of 0.3 mL during all of the trial period. The mice were intraperitoneally treated with ENU (50 mg/kg p.c.) on the 15th day; the mice were sacrificed on the next day (16th day) by decapitation followed by collecting cells from the bone marrow.
Group 6 - Group treated with NP_{EPV} (1 mg/mL) via gavage with administration of 0.3 mL during all of the trial period. The mice were intraperitoneally treated with ENU (50 mg/kg p.c.) on the 15th day; the mice were sacrificed on the next day (16th day) by decapitation followed by collecting cells from the bone marrow.
Group 7 - Group treated with LP_{EPV} (1 mg/mL) via gavage with administration of 0.3 mL during all of the trial. The mice were intraperitoneally treated with 0.9% NaCl (0.1

mL/10g p.c.) on the 15th day; the mice were sacrificed on the next day (16th day) by decapitation followed by collecting cells from the bone marrow.

Group 8 - Group treated with NP_{EPV} (1 mg/mL) via gavage with administration of 0.3 mL during all of the trial period. The mice were intraperitoneally treated with 0.9% NaCl (0.1 mL/10g p.c) on the 15th day; the mice were sacrificed on the next day (16th day) by decapitation followed by collecting cells from the bone marrow.

Group 9 - Group only treated with free *Pyrostegia venusta* leaf extract (EPV, 1 mg/mL) via gavage with administration of 0.3 mL during all of the trial period. The mice were intraperitoneally treated with ENU (50 mg/kg p.c.) on the 15th day; the mice were sacrificed on the next day (16th day) by decapitation followed by collecting cells from the bone marrow.

Biochemical analysis

Serum levels for hepatic activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes, as well as creatinine (CRT) in order to assess the renal activity were measured by Cobas Integra 400 Plus (Roche) equipment through the chemiluminescence method.

Statistical analysis

The frequency of micronucleated cells in the different trial groups was compared by the Chi-squared test (Pereira, 1991). The Tukey test with $p<0.05$ being considered statistically significant was used for the biochemical analysis in the SISVAR statistical program.

RESULTS

The nanostructured formulations (LP_{EPV} and NP_{EPV}) had a homogeneous macroscopic appearance, milky aspect and opalescent and slightly greenish color, which were expected characteristics due to the *P. venusta* extract. The LP_{Bl} and NP_{Bl} formulations had a whitish color. All formulations had blue reflex caused by the Brownian motion of the nanosystems in the aqueous medium.

Table I presents the values of photon correlation spectroscopy (PCS) analysis of three batches of each formulation, showing monomodal particle size distributions with **z**-average diameters between 203 ± 3.61 nm and 293 ± 6.85 nm. As the PCS analyzes were performed with 11 scans of 10 s each, it is possible to infer the kinetic stability of the colloids (Fiel *et al*., 2013). The polydispersity index (PDI) was lower than 0.2 for all formulations, and the results are consistent with studies on nanosystems (Bitencourt *et al*., 2016; Frank *et al*., 2019). The nanometric diameters of the formulations was confirmed by laser diffraction which demonstrated low polydispersion demonstrated by the range values, which indicates homogeneity in the samples. Zeta potential showed the presence of the surface charge potential of the nanosystems by electrophoretic mobility through the steric blockage of the nanostructures, with ξ near zero. A significant difference was observed in the potential comparison between NP and LP formulations, being considered very distinct formulations. Negative values are due to functional groups which contain oxygen present on the colloid surface and consequently have negative partial density at the pseudo-phase interface. The pH of LP_{Bl} and LP_{EPV} was neutral and NP_{Bl} and NP_{EPV} more acid, consistent with the composition of the formulations.

TABLE I - Nanostructure characterization along 30 days of study

Formulation	d4.3 (μm)	Span	pH	Zeta Potential (ζ) (mV)
LP_{Bl}	0.29±0.00	0.97±0.00	7.28 ± 0.03	-16.9± 0.37
LP_{EPV}	0.36±0.00	0.71±0.11	7.25 ± 0.05	-16.5± 0.26
NP_{Bl}	0.35±0.01	0.97±0.11	5.68 ± 0.17	-5.96±0.25
NP_{EPV}	0.34±0.00	0.90±0.00	4.95 ± 0.14	-11.9± 0.21

LP_{Bl} – White liposome; LP_{EPV} – Liposome containing extract; NP_{Bl} – White polymeric nanocapsule; NP_{EPV} – Polymeric nanocapsule containing extract.

There were five flavonoids (apigenin, luteolin, myricetin quercetin 3-β-d-glycoside and rutin) identified by LC-MS/MS in the *P. venusta* ethanolic extract (Figure 1) and demonstrated in Table II below.

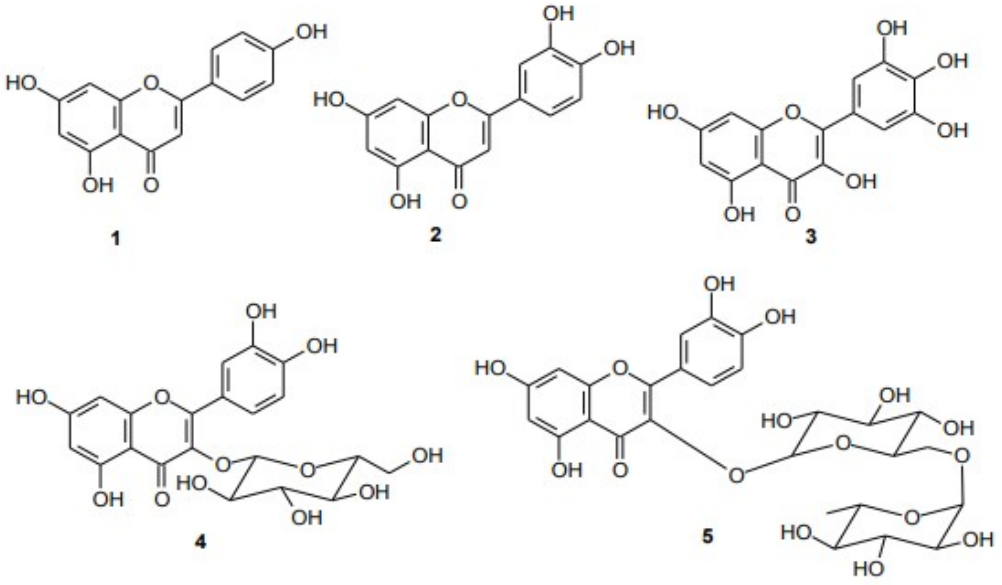

FIGURE 1 - Structures of the flavonoids identified in ethanolic extract from *P. venusta*.

TABLE II - LC-MS/MS parameters of the five flavonoids identified in the *Pyrostegia venusta* crude ethanolic extract

Compound	$[M–H]^-$	T.R. (min)	MRM Transition	Molecular formula
1 Apigenin	269.4	16.6	269.24→ 116.80	$C_{15}H_{10}O_5$
2 Luteolin	285.24	14.9	285.24→133.00	$C_{15}H_{10}O_6$
3 Myricetin	217.24	9.5	217.24→137.00	$C_{15}H_{10}O_8$
4 Quercetin-3-β-d-glycoside	463.38	10.8	463.38→300.00	$C_6H_{11}O_5$
5 Rutin	609.27	10.4	609.27→300.20	$C_{27}H_{30}O_{16}$

$[M – H]^-$ - molecular ion; T.R. (min) - retention time; MRM Transition - Multiple reaction tracking.

Table III presents the micronucleus frequency in polychromatic erythrocytes (PCEMNs) after pre-treatment with nanostructured and free *P. venusta* ethanolic extract. The results show 183% reduction in the frequency of PCEMNs for the LP_{EPV} + ENU group ($p<0.001$) and 114% for the NP_{EPV} + ENU group ($p<0.001$) if compared to their respective positive controls LP_{Bl} + ENU and NP_{Bl} + ENU, suggesting significant antimutagenic activity of the nanostructured extract, in addition to not showing a significant increase of PCEMNs if compared to the negative controls (LP_{Bl} + 0.9% NaCl and NP_{Bl} + 0.9% NaCl).

TABLE III - Frequency of micronuclei in polychromatic erythrocytes (MNPCEs) of bone marrow of mice after pre-treatment with *Pyrostegia venusta* ethanolic extract

Treatment	Number of PCEs analyzed	PCEMNs		Reduction %
		No.	%	
LP_{Bl} + NaCl 0.9 % a	6000	234	3.90	
NP_{Bl} + NaCl 0.9 %[a]	6000	229	3.82	
LP_{Bl} + ENU (50 mg/kg) [b]	5000[c]	371	7.42	
NP_{Bl} + ENU (50 mg/kg) [b]	6000	411	6.85	
LP_{EPV} + ENU (50 mg/kg)	6000	120	2.00	183*
NP_{EPV} + ENU (50 mg/kg)	6000	204	3.40	114*
LP_{EPV} + NaCl 0.9 %	6000	161	2.68	
NP_{EPV} + NaCl 0.9 %	6000	241	4.02	
EPV + ENU	6000	266	4.43	

[a] Negative control; [b] Positive control; [c] 1 animal died; * $p < 0.001$ for the Qui-square test.

Table IV presents the feed consumption (g/week/group) and body weight (g) during the animal treatment period. The results suggest that the treatments did not cause toxicity to the animals, since there was no interference in the feeding nor to the weight gain.

TABLE IV - Feed average consumption (g/week/group) and body weight (g) of mice treated during 15 days with *Pyrostegia venusta* ethanolic extract

Treatment	Number of animals	Feed average consumption (g/week/group)	Body weight (g)
LP_{Bl} + NaCl 0.9% [a]	6	33.74 [d]	35.80 [d]
NP_{Bl} + NaCl 0.9% [a]	6	37.93 [d]	36.35 [d]
LP_{Bl} + ENU (50 mg/Kg) [b]	5 [c]	35.29 [d]	33.79 [d]
NP_{Bl} + ENU (50 mg/Kg) [b]	6	35.83 [d]	32.36 [d]
LP_{EPV} + ENU (50 mg/Kg)	6	32.08 [d]	33.41 [d]
NP_{EPV} + ENU (50 mg/Kg)	6	35.65 [d]	34.10 [d]
LP_{EPV} + NaCl 0.9%	6	38.40 [d]	34.68 [d]
NP_{EPV} + NaCl 0.9%	6	33.93 [d]	35.21[d]
EPV + ENU (50 mg/Kg)	6	35.49 [d]	32.99 [d]
C.V. (%)	-	9.72	8.30
Fc	-	0.23^{NS}	0.14^{NS}

[a] Negative control; [b] Positive control; [c] 1 animal died; [d] Averages followed by the same letter do not differ among each other, by Tukey Test (α= 5 %); [NS] Not significant; C.V. = coefficient of variance; Fc = correction factor.

Table V presents transaminases (AST and ALT) and creatinine dosages regarding the possible toxicity in the administration of *P. venusta* ethanolic extract during the trial period. The results show that there was no significant difference among the treated groups, demonstrating that there were no hepatic or renal damage regarding *P. venusta* administration.

TABLE V - Biochemical analysis of the hepatic (AST and ALT) and renal (CRT) activities of control and treated with *Pyrostegia venusta* ethanolic extract groups during 15 days

Groups	AST	ALT	CRT
LP_{Bl} + NaCl 0.9 %	67.78 [a]	288.80 [a]	0.21 [a]
NP_{Bl} + NaCl 0.9 %	77.06 [a]	316.08 [a]	0.28 [a]
LP_{Bl} + ENU (50 mg/Kg)	60.80 [a]	275.07 [a]	0.19 [a]
NP_{Bl} + ENU (50 mg/Kg)	62.00 [a]	327.58 [a]	0.29 [a]
LP_{EPV} + ENU (50 mg/Kg)	50.67 [a]	249.82 [a]	0.20 [a]
NP_{EPV} + ENU (50 mg/Kg)	53.83 [a]	199.30 [a]	0.20 [a]
LP_{EPV} + NaCl 0.9 %	66.92 [a]	259.52 [a]	0.21 [a]
NP_{EPV} + NaCl 0.9 %	66.65 [a]	279.65 [a]	0.20 [a]
EPV + ENU (50 mg/Kg)	65.87 [a]	223.73 [a]	0.31 [a]
C.V. (%)	21.15	25.35	52.30
Fc	1.42^{NS}	1.17^{NS}	0.69^{NS}

[a] Averages followed by the same letter do not differ among each other, by Tukey Test (α= 5 %); [NS] Not significant; C.V. = coefficient of variance; Fc = correction factor.

DISCUSSION

The developed formulations showed a homogeneous macroscopic aspect of whitish color if formulated without *P. venusta* ethanolic extract (LP_{Bl} and NP_{Bl}). This result is in accordance with the appearance of the formulations and consistent with what is described in the literature for these types of nanostructured systems (Fiel *et al*., 2013; Bittencourt *et al*., 2016). On the other hand, other formulations showed a milky greenish aspect after extract incorporation (LP_{EPV} and NP_{EPV}) and a fresh green leaf smell, characteristic of *P. venusta* ethanolic extract.

The suspension diameter evaluations demonstrated nanometer particle sizes between 200 and 300 nm. The systems presented low polydispersion as evaluated by Dynamic light scattering and confirmed by laser diffraction (Frank *et al*., 2019).

A cationic group in the formulation resulting from phosphatidylcholine provided a neutral pH to the liposomes (Perttu, Kohli, Szoka, 2012). On the other hand, the pH of the nanoparticles suspensions was in accordance with Ribeiro *et al*. (2016), shown by the authors around pH 5.44. This NP characteristic is due to the presence of biodegradable polymer (PCL) with terminal carboxylic acid groups and therefore reduces the pH (Ribeiro *et al*. (2016). The ζ produces electrostatic repulsion among its neighbor colloids, being extremely relevant since it avoids agglomeration (Perttu, Kohli, Szoka, 2012).

The bioactive compounds from plant extracts show difficulty in intestinal absorption due to their low lipid solubility and the use of nanosystems improves this absorption by oral administration (v.o). These carriers enhance and optimize the ability of compounds such

as flavonoids to enter and store within tumor tissues, improving the permeability effect and optimizing serum levels. In addition to enhancing the prevention of genetic damage, therapeutic doses of compounds extracted from plants are intensified through changes in their structures to improve their affinity in the body (Dong, Mumper, 2010).

The results show that the use of nanosystems suggests an improvement in the bioavailability of compounds from *P. venusta* leaf extract and also demonstrates better biological activity by increasing the absorption of the components. A significant reduction in the frequency of micronucleus in polychromatic erythrocytes was observed for the mice treated with *P. venusta* nanostructured extract for both nanosystems used.

The higher potentiation of the antimutagenic effect of *P. venusta* occurred when carried in liposomes, as it is believed that nanocarriers improve the digestive system absorption, as well as improve bloodstream stability and metabolism of this extract (Dong, Mumper, 2010). On the other hand, there are no studies of *P. venusta* related to its protective effect against genotoxic agents by using polymeric nanocapsules, making this study an original work about the identification of protective effect of nanostructured *P. venusta* for cancer prevention, suggesting an effective antioxidant activity of the flavonoids in this specie.

Both the liposome and polymeric nanoparticle nanosystems protect phytochemical compounds against obstacles such as physical and chemical degradation, biotransformation processes, withstanding the unexpected and abrupt degradation (Nair *et al*., 2010). The advantage of using liposomes is mainly due to their characteristic phospholipidic bilayer, which improves the likely limitations of the poor bioavailability of flavonoids, for example. This turns their ability to avoid degradation into a perspective for their use in anticancer therapy (Lim, Lee, Kim, 2004). Moreover, their efficient protection is also related to the ability of liposomes of being captured by macrophages, increasing their concentration in liver, spleen and bone marrow (Frézard *et al*., 2005).

In accordance with Ferreira *et al*. (2009), the chemopreventive activity over the DNA may be linked to flavonoids, which act as potent antimutagenic agents due to their antioxidant activity. In this context, the following flavonoids were identified in the *P. venusta* leaf ethanolic extract: apigenin (1), luteolin (2), myricetin (3), quercetin-3-β-d-glycoside (4) and rutin (5).

Quercetin has also been identified by Roy *et al*. (2011) in *P. venusta* flowers and roots and by Veggi, Cavalcanti and Meireles (2014) in leaves. Pereira *et al*. (2014) found rutin in *P. venuta* flowers. Apigenin (1) and luteolin (2) were found in other species of the Bignoneaceae family, however this is their first report in *P. venusta* (Zoghbi, Oliveira, Guilhon, 2009). According to Blatt, Santos and Salatino (1998), rutin seems to be easily found in species cultivated in Brazilian cerrado, as these authors identified this flavonoid in *P. venusta* leaves. The identification of myricetin (3) in the Bignoneaceae family is also unprecedented (Pereira *et al*., 2014).

The antioxidant activity of *P. venusta* leaves had been previously observed in *in vitro* tests by Altoé *et al*. (2014). Pereira *et al*. (2014) verified the antioxidant activity of phenolic compounds in *P. venusta* flower free extract. Khan, Afaq and Mukhtar (2008) reported that agents able to interfere in more than one crucial point in the carcinogenesis process may act in blocking the attachment of an injury in the DNA for cancer prevention, showing an advantage over the single-target agents.

It is known that quercetin acts on the cellular cycle regulation and is able to induce apoptosis in tumor cells, in addition to inhibiting tyrosine kinase activity (Dornas *et al*., 2007). Apigenin and quercetin act together by preventing cancer through a proteasome inhibition mechanism selective to cancer which does not affect normal cells, contributing to preventive effects of the disease (Chen *et al*., 2005). Luteolin adds action to the other flavonoids inducing the apoptosis of tumor cells by interrupting the cellular cycle in the G0/G1 phase, which may be the mechanism which luteolin uses to reduce the cellular viability of damaged cells (Cao *et al*., 2017). Apigenin has the ability to promote dissipation of absorbed energy, increasing its action level as antioxidant defense (Gobbo-Neto, Lopes, 2007).

Therefore, it can be suggested that the antimutagenic effect observed by *P. venusta* leaf ethanolic extract might be a result of the synergism among the plant compounds, with a mechanism of action which has not yet been elucidated.

Regarding the genotoxic evaluation, the LP_{EPV}, NP_{EPV}, LP_{Bl}, NP_{Bl} and EPV nanosystems did not show mutagenic effect. In studying free *P. venusta* flower extract, Magalhães *et al*. (2010) evaluated the clastogenic effects in different concentrations (50, 100 and 200 mg/kg p.c) in Swiss mice by micronucleus test and did not verify a significant increase in the average number of cells with micronucleus or chromosomal defects.

The administration of free or nanostructured *P. venusta* extract also did not show a physical sign of toxicity in animals during the trial period such as weight loss, lack of water ingestion or loss of appetite. The data shows a weight gain during treatment, which is considered normal (Spinelli *et al*., 2012).

Furthermore, no hepatotoxic and nephrotoxic effects were observed from the AST, ALT and CRT dosages, respectively. The use of inert nanocarriers containing *P. venusta* extract did not demonstrate possible hepatic damage, confirming the ability of nanostructures to protect against undesirable interactions (Lim, Lee, Kim, 2004; Spinelli *et al*., 2012).

Finally, according to Roy *et al*. (2011) and Mostafa, El-Dahshan and Singab, (2013), *P. venusta* has compounds with great potential in traditional medicine and in the discovery of new products with pharmacological potential.

CONCLUSION

In conclusion, the developed nanostructures had nanometric size with monomodal characteristic and physico-chemical stability compatible with the literature and the observed effects. identified total of five flavonoids with potent antioxidant activity were identified in the *P. venusta* leaf ethanolic extract, suggesting that the extract contains compounds which act in the prevention or neutralization of DNA damage induced by the chemical agent *in vivo*. In this context, there was antimutagenic activity potentiation of *P. venusta* extract carried in liposomes and polymeric nanoparticles when compared with the activity in the free form by the micronucleus test and without possible harmful effects to the mice during the experimental period. Thus, under the trial conditions, nanostructured *P. venusta* leaf ethanolic extract, blank nanostructures and the free extract did not present possible mutagenic, hepatotoxic or nephrotoxic effects in the mice, showing great pharmacological potential for cancer chemoprevention.

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