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Pharmacotoxic evaluation of extracts of medicinal plants used in the treatment of obesity

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Medicinal plants are rich sources of bioactive compounds with potential for therapeutic, cosmetic and food use, and many of them are consumed only based on popular use, without any scientific proof of their efficacy and safety. The objective of this study was to evaluate the toxic and pharmacological effects of aqueous extracts of the plant species *Aloe vera* (L.) Burm., *Simaba ferruginea* St. Hil., *Baccharis trimera* (Less.) DC, *Garcinia cambogia* Desr. and *Tournefortia paniculata* Cham., popularly used as auxiliaries in the treatment of obesity, in order to obtain information on the safety of its use. The genotoxic, hemolytic, phospholipase, coagulant and fibrinogenolytic effects were evaluated. It was observed that, at concentrations of 125 and 250 µg/ml, the plant extracts showed no genotoxic, phospholipase, hemolytic (except for *B. trimera*), coagulant and fibrinogenolytic potential. Although no genotoxicity and erythrocyte or phospholipid lysis was observed, defining what is safe regarding the use of these extracts depending on the implementation of comprehensive preclinical and clinical characterizations.

Key words: Toxicity, obesity, medicinal plants.

INTRODUCTION

The incidence of overweight and obesity has been increasing worldwide, and is considered a global epidemic, characterized as a major contributor to the high prevalence of chronic and disabling diseases nowadays. It is estimated that there are over one billion overweight adults in the world and, among them, 400 million are clinically obese (Tucci et al., 2010).

Due to the side effects and the high cost of drugs traditionally used in the treatment of obesity, the potential of natural products has been widely explored, and they may be a viable alternative for the future development of drugs that effectively and safely induce weight reduction (Mayer et al., 2009). Studies show that several natural products, including extracts and compounds isolated from plants, are used for reducing body weight and preventing obesity (Souza et al., 2011; Simão et al., 2012).

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Medicinal plants are important due to their contribution as natural drug sources and to the fact that they provide great chances for the obtainment of prototype molecules, owing to the wide diversity of their constituents (Yunes and Calixto, 2001). However, many plants are used in phytotherapic preparations without suitable quality control and security, considering that scientific literature shows, for most medicinal plants, the presence of toxic substances and/or a variable chemical composition (Capasso et al., 2000).

Several studies have reported the presence of many secondary metabolites with toxic, genotoxic and carcinogenic potential when used chronically or acutely (Ernest, 2004; Rietjens et al., 2005). However, toxicological studies have been conducted very slowly, resulting in inconclusive data on the effects of medicinal plants on human health.

According to Nunes and Araújo (2003), the use of plants in folk medicine is a practice carried out for centuries to cure diseases, and their preparation and proper use bring many benefits. However, studies on their pharmacological and toxicological effects in humans are still scarce for most medicinal plants/phytotherapics with possible activity in the treatment of obesity.

In this context, plants that are popularly used for therapeutic purposes, but lack scientific evidence of their effects, are of great value in the search for the development of new drugs of proven efficacy and safety.

Infusions, decoctions and teas of *Aloe vera* (L.) Burm. (aloe), *Simaba ferruginea* St. Hil. (calunga), *Baccharis trimera* (Less.) DC (carqueja), *Garcinia cambogia* Desr. and *Tournefortia paniculata* Cham. (marmelinho) are widely used in Brazilian folk medicine, alone or associated with each other, as auxiliaries in the treatment of obesity for which there are no studies related to its therapeutic action or toxicological properties, highlighting the importance of research that aims to improve the pharmacological and toxicological characterization of these plants (Simão et al., 2012, 2013).

The objective of this study was to evaluate the genotoxic, hemolytic, coagulant, phospholipase and fibrinogenolytic potential of aqueous extracts of the medicinal plant species *A. vera*, *S. ferruginea*, *B. trimera*, *G. cambogia* and *T. paniculata*, in order to predict safety levels for their use in the treatment of obesity and suggest new perspectives of therapeutic use.

MATERIAL AND METHODS

Plant

B. trimera and *T. paniculata* leaves, as well as the stem bark of *S. ferruginea*, were acquired in the municipal market of Belo Horizonte, Minas Gerais, in January 2011. *B. trimera* and *T. paniculata* leaves were washed with tap and distilled water, and then placed together with the stem bark obtained from *S. ferruginea* in forced air circulating ovens to dry for 48 h, at 35°C. After drying, the leaves and bark were ground in a Wiley-type mill. The commercial powder of *A. vera*, obtained by lyophilization of the plant mucilage, and of *G. cambogia*, obtained by spray drying, were acquired from FLORIEN, a distributor of pharmaceutical raw materials.

Preparation of plant extracts

The plant flours were mixed with distilled water in the proportion 1:100 (w/v), and placed on a horizontal shaker (100 rpm) at room temperature for 5 min. They were then centrifuged at 5,000 × g for 5 min. The supernatant was collected and used in the tests described subsequently. This ratio was chosen considering information about the use of dry leaves for the preparation of teas, infusions and decoctions in Brazilian folk medicine for human consumption, and is called usual dose.

Comet assay

Obtainment of human blood and preparation of treatments

The peripheral blood of five volunteers aged between 21 and 40 years (members of our research team) was used, after free informed consent; the volunteers did not present disease symptoms and reported not having used prescription drugs for at least 30 days prior to collection. The blood was collected in vacuum tubes containing heparin and immediately protected from light; the entire experiment was conducted in accordance with the standards of the Ethics Committee on Human Research (COEP) of Universidade Federal de Lavras. The plant extracts at concentrations of 125 and 250 µg/ml were then added to 500 µl aliquots of blood diluted in 500 µl of phosphate-buffered saline (PBS).

The concentrations chosen for the evaluation of the plants extracts correspond to the equivalent dose and twice the dose recommended for human consumption of teas, infusions and decoctions, seeking weight loss, considering the equivalency between the blood volumes of an adult individual and that used in the assay. Negative (without treatment) and positive (10 µg/ml doxorubicin) controls were conducted simultaneously. The blood samples were incubated in an oven at 37°C, in the presence of the treatments for 4 h. The comet assay was then performed.

Obtainment of nucleoids and electrophoretic run

The comet assay was performed according to the methodology described by Singh et al. (1988), with some modifications. Therefore, an aliquot (15 µl) of each cell suspension containing the treatments was mixed with 100 µl of low melting point agarose (0.5% w/v in PBS), applied to a microscope slide previously coated with standard agarose solution (1% w/v in PBS), immediately overlaid with a coverslip and kept at ±4°C for 5 min.

For each treatment/volunteer, 3 slides were prepared. Then, the coverslips were removed and the slides were immersed in lysis solution (2.5 mol/L NaCl, 100 mmol/L ethylenediaminetetraacetic acid (EDTA), 10 mmol/L Tris, 1% Triton X-100, 10% dimethyl sulfoxide (DMSO); pH 10), where they remained for 24 h at 4°C, allowing the removal of anucleate cells and plasma molecules, as well as the lysis of lymphocytes, in order to isolate only their nucleoids for analysis.

After lysis, the slides were kept at ± 10°C for 25 min in a freshly prepared electrophoresis solution (1 mmol/L EDTA, 300 mmol/L NaOH; pH 13), in order to expose the alkali-labile sites, and then subjected to electrophoresis, conducted at 25V for 35 min. After the electrophoresis, the slides were kept in a neutralization solution (0.4 mol/L Tris; pH 7.5) for 30 min, and then dried and fixed with 100% ethanol. All procedures were performed in the dark.

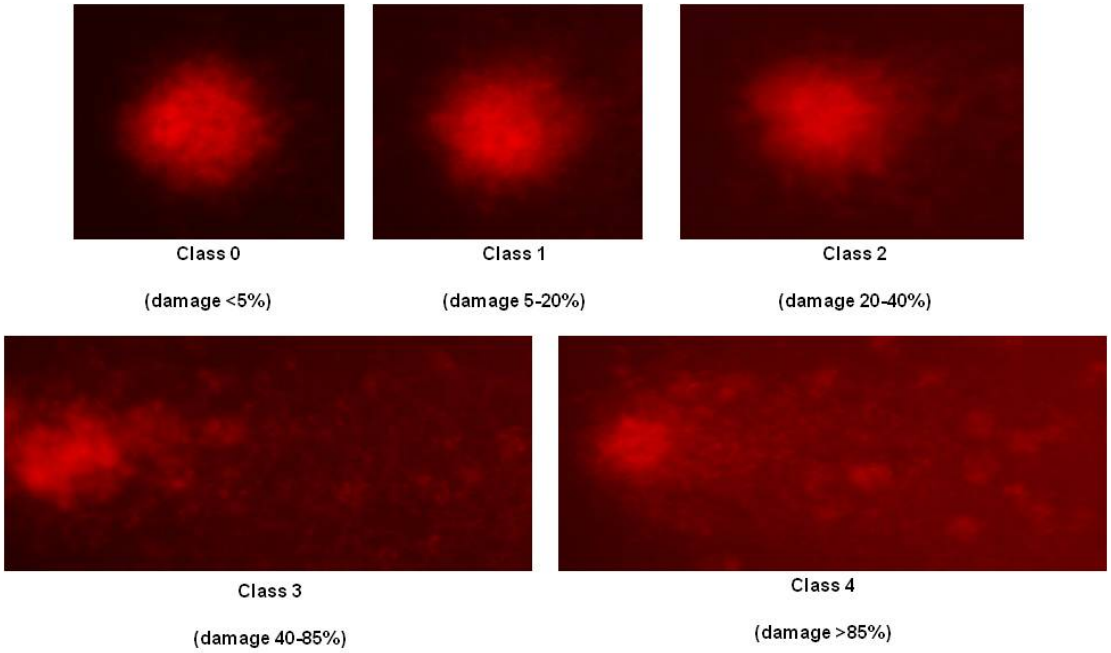

Figure 1. Nucleoid photomicrographs of human lymphocytes treated with doxorubicin and subjected to the electrophoretic run on agarose gel, classified as representing comets of the various fragmentation levels of the DNA molecules. These images correspond to the visual score used by the evaluator during the analysis of the slides in epifluorescence microscope at 400x magnification.

Staining and analysis

The slides were stained with 45 µl of propidium iodide solution (1 mg/ml), overlaid with a coverslip and analyzed in an epifluorescence microscope (Nikon ECLIPSE E400) at 200 and 400X magnifications.

In order to measure damage levels in lymphocyte DNA molecules, 100 nucleoids of each slide, 3 slides per treatment/ individual (totaling 300 nucleoids per treatment/individual), were counted and classified by the same evaluator, using visual score patterns described by Singh et al. (1988).

The cells were classified according to the size of the "tail" and the diameter of the "head" in Class 0: no damage (damage<5%); Class 1: low damage level (5 to 20%); Class 2: intermediate damage level (20 to 40%); Class 3: high damage (40 to 85%); Class 4: totally damaged (damage>85%) (Figure 1). The average frequency of damage was calculated from the sum of the percentages of damage 1, 2, 3 and 4. The arbitrary units (0 to 400; where 0 = no damage and 400 = 100% damage) were calculated by the equation (1 × damage in class 1) + (2 × damage in class 2) + (3 × damage in class 3) + (4 × damage in class 4), as described by Collins (2004).

Lysis of phospholipids

The phospholipase activity was evaluated by the use of solid medium, as described by Gutiérrez et al. (1988), in which there was a modification, e.g. the exchange of agarose for agar, reducing the costs of the assay. A gel was prepared (0.01 mol/L $CaCl_2$; egg yolk 1:3 v/v PBS; 0.005% sodium azide) with 1% bacteriological agar, pH 7.2 and, after its solidification, uniform-sized holes were made (~0.5 cm diameter) and the plant extracts (125 and 250 µg/ml) were applied in a final volume of 25 µl.

The gels containing the samples were kept in an oven at 37°C for 12 h, and the formation of a translucent halo around the hole in the gel characterized phospholipase activity, which is quantified by measuring the halo diameter. The assays were performed in triplicate.

Hemolysis of human erythrocytes

Solid medium

For the evaluation of hemolysis of human erythrocytes in solid medium, the same methodology described earlier was used, substituting the egg yolk for erythrocytes in PBS solution. The blood was collected in vacuum tubes containing sodium citrate, immediately centrifuged at 400 × g (Fanem Baby®I Model 206 BL) for 10 min and subjected to 3 washes in the same volume of PBS, obtaining erythrocytes at 100% hematocrit. After the first centrifugation, plasma was collected and stored for use in the coagulation tests.

Liquid medium

For the evaluation of hemolysis in liquid medium, 10 ml of blood were centrifuged at 900 × g for 5 min. The plasma was removed and the red cells were suspended in 5 mmol/L phosphate buffer, pH 7.4, and then centrifuged under the same conditions, repeating this washing procedure 3 times at 4°C. The 100% red blood cell concentrate was diluted to 0.15% hematocrit (13 µmol/L lipid), using the same buffer (Preté et al., 2011).

The hemolytic activity was evaluated by incubating 1 ml of erythrocyte suspension (0.15%) for 60 min at 37°C with the plant extracts at the concentrations of 125 and 250 µg/ml, followed by centrifugation at 2,500 × g for 5 min and determination of the hemoglobin concentration in the supernatant by spectrophotometry at 412 nm (Shimadzu UV-160 1 PC). The controls were performed

with erythrocyte suspension in PBS (c_1 = mechanical hemolysis control) and distilled water (c_2 = total hemolysis control) (Preté et al., 2011).

Hemoglobin concentration was determined using the equation:

$$\text{Hemolysis (\%)} = \frac{A_a - A_{c1}}{A_{c2} - A_{c1}} \times 100$$

where A_a, A_{c1} and A_{c2} are, respectively, the absorbance of the sample and of the controls c_1 and c_2 at 412 nm.

Actions on the coagulation process of human blood plasma

The coagulant activity was performed using citrated human plasma (200 µl) stabilized at 37°C, proceeding with the addition of the plant extracts at the concentrations of 125 and 250 µg/ml, or venoms of *B. moojeni* at a dose of 20 µg, followed by timing. Venoms were evaluated as inducers of blood clot formation, standardized at a range between 1 and 5 min (Selistre de Araújo et al., 1990). The plasma and the extracts incubated were observed for 120 min, allowing the certification of absence of coagulating action.

Degradation of bovine fibrinogen

According to the methodology described by Rodrigues et al. (2000), different extract concentrations (100 and 200 µg/ml) were incubated with bovine fibrinogen (80 µg) at 37°C for 60 min. The samples were analyzed by 12% polyacrylamide gel electrophoresis under denaturing conditions (Laemmli, 1970), allowing the observation of α, β and γ chains of the fibrinogen control, as well as the presence of fibrinopeptides in the samples in which proteolysis occurs.

Statistical analysis

The results of the comet assay were statistically evaluated by analysis of variance and the means were compared using the Scott Knott test ($P<0.05$) with the aid of the R software (R Development Core Team, 2011). For this test, Arbitrary Units were also calculated with predetermined values between 0 and 400, respectively representing absence of damage and 100% damage, as described by Collins (2004). For the other assays, data were treated and stored as means and standard deviation of triplicates.

RESULTS AND DISCUSSION

In the results of fragmentation of DNA molecules (Table 1), a predominance of nucleoids with no damage was observed (class 0), with percentage averages ranging between 19.33% (*S. ferruginea*, 125 µg/ml) and 56.19% (*G. cambogia*, 250 µg/ml), and with low damage levels (class 1), with averages between 43.13% (*G. cambogia*, 250 µg/ml) and 74.81% (*S. ferruginea,* 125 µg/ml). A reduced number of nucleoids was observed in class 2 (1.16% for *A. vera*, 125 µg/ml, and 5.83% for *B. trimera*, 250 µg/ml). In relation to class 3 (averages ranging between 0 and 0.97%) and class 4 (averages ranging between 0 and 0.27%), corresponding to high levels of damage and totally damaged DNA, respectively, there was no statistical difference between the nucleoids treated with plant extracts and the untreated ones.

Doxorubicin is an antitumor agent of the anthracycline family, capable of causing DNA damage and, therefore, is widely used as a control in the comet assay, since it induces damage equal or superior to 40% in animal cells (Barcelos et al., 2009). It led to values of 34.46, 22.88, 20.24, 10.48 and 11.97%, for classes 0 to 4, respectively, highlighting its potential in inducing DNA fragmentation, even when used at low doses (Table 1).

The average frequency of damage (Table 1) shows that, in general, there was no significant increase in the amount of damaged nucleoids with increased concentrations of plant extracts. The value of arbitrary units obtained for the treatment with doxorubicin was 3 times higher than that obtained for the negative control, 1.6 times higher than the highest value obtained for the treatments with the extracts of *S. ferruginea* and 3.07 times higher than the lowest value obtained for the extracts of *G. cambogia*. It is necessary to point out that doxorubicin was used at concentrations of 12.5 and 25 times smaller than those used for the extracts, which suggests the absence of genotoxic potential for the extracts under the tested conditions: 4 h of incubation of the extracts with the cells; blood volume of 500 µl and concentrations of 125 and 250 µg/ml.

The values of damage frequency (%) correspond to the sum of nucleoids which are not intact, that is, any fragmentation of DNA molecules, even small, will contribute to increase these values. Thus, it is possible to observe a proximity between the values for the genotoxic drug and those assigned to treatments with different plants. However, the values of arbitrary units define the genotoxic potential more precisely, since the formula used provides fragmented DNA molecules with a higher value to an increasing degree, that is, for classes 3 and 4 (relating to the highest fragmentation levels), the numbers of nucleoids are multiplied by 3 and 4, respectively.

Studies performed with aqueous extracts of *A. vera* show that, at usual doses (40 ml/L), the aqueous extract of this plant does not induce mutagenicity in *Allium cepa*, whereas, at higher doses than the ones commonly used, the extract was capable of inducing cytotoxicity and mutagenicity, when evaluated on the same cell type. In this study, the authors also found an antimutagenic effect (mutagenicity induced by paracetamol) for the *A. vera* gel on human lymphocytes, using the micronucleus test (Sturbelle et al., 2010), which was also verified by Stanic (2007) in a study on the antimutagenic action of the *A. vera* gel on the effects induced by ethyl methanesulfonate.

Rodrigues et al. (2009), when analyzing aqueous extracts of *B. Trimera* administered to mice by gavage at doses of 200, 500 and 2,000 mg/kg for 3 days, found no genotoxic effects in blood and liver samples from these animals by the comet assay. However, the aqueous extract showed an antigenotoxic potential (oxidative

Table 1. Average nucleoid number per class of comet, frequency of nucleoids with damage and arbitrary units after the treatment with aqueous extracts of medicinal plants used in the treatment of obesity.

Treatment (µg/ml)		Class of comet (%)[1]					Frequency of damage (%)[2]	Arbitrary units[3]
		0	1	2	3	4		
Negative control		53.76±16.66[a]	45.06±12.90[b]	1.20±0.20[c]	0.00±0.00[b]	0.00±0.00[b]	46.26±13.37[b]	47.46±14.85[c]
Positive control[4]		34.46±2.15[b]	22.88±2.22[c]	20.24±1.10[a]	10.48±1.18[a]	11.97±1.44[a]	65.54±3.54[a]	142.68±8.56[a]
Aloe vera	125	36.66±4.55[b]	62.93±8.39[a]	1.16±0.06[c]	0.00±0.00[b]	0.00±0.00[b]	64.09±8.03[a]	65.25±7.74[b]
	250	30.03±5.57[b]	68.39±6.54[a]	1.58±0,09[c]	0.00±0.00[b]	0.00±0.00[b]	69.97±5.37[a]	71.55±4.41[b]
Simaba ferruginea	125	19.33±4.57[b]	74.81±5.94[a]	5.33±0.52[b]	0.33±0.04[b]	0.20±0.02[b]	80.67±6.43[a]	87.26±10.56[b]
	250	29.26±14.17[b]	68.13±11.13[a]	2.66±0.40[c]	0.00±0.00[b]	0.00±0.00[b]	70.74±11.38[a]	73.45±11.64[b]
Baccharis trimera	125	30.95±11.03[b]	65.03±16.69[a]	2.66±0,55[c]	0.00±0.00[b]	0.00±0.00[b]	60.81±16.94[a]	76.17±21.61[b]
	250	39.19±15.66[b]	54.83±19.74[a]	5.83±0.37[b]	0.13±0.01[b]	0.00±0.00[b]	69.81±25.66[a]	66.06±32.00[b]
Garcinia cambogia	125	26.66±9.20[b]	72.73±21.23[a]	0.66±0.03[c]	0.00±0.00[b]	0.00±0.00[b]	73.40±21.02[a]	74.05±20.81[b]
	250	56.19±11.88[a]	43.13±12.24[b]	1.64±0.24[c]	0.00±0.00[b]	0.00±0.00[b]	43.81±12.42[b]	46.39±12.73[c]
Tournefortia paniculata	125	36.13±4.07[b]	61.95±3.67[a]	1.73±0,06	0.13±0.01[b]	0.06±0.04[b]	63.87±3.91[a]	66.06±5.62[b]
	250	28.56±4.41[b]	68.60±5.81[a]	1.60±0.16[c]	0.97±0.05[b]	0.27±0.03[b]	71.44±5.55[a]	75.75±5.40[b]

[1]Data represent the mean values obtained for 300 nucleoids/treatment/volunteer. 5 volunteers were used (1 volunteer/experiment); [2]Frequency of damage: sum of the damage of classes 1 to 4; [3]Arbitrary units: (1 × damage in class 1) + (2 × damage in class 2) + (3 × damage in class 3) + (4 × damage in class 4), according to the methodology described by Collins (2004); [4]Positive control: 10 µg/ml doxorubicin; Same letters in the columns do not differ by the Scott-Knott test (P<0.05).

damage induced by hydrogen peroxide) in blood samples from these mice by the comet assay, which may be due to the antioxidant potential demonstrated *in vivo* by the aqueous extract of this plant, and can be responsible for protecting DNA molecules against oxidative damage induced by free radicals.

Baggio et al. (2003), when analyzing aqueous and hydroalcoholic extracts of *B. trimera* leaves administered for 24 h at doses from 1.0 to 6.0 g/kg, and Dias et al. (2009), analyzing the lyophilized crude extract of this plant administered for two weeks at dose 5 g/kg, did not observe cytotoxic effects in Wistar rats, which was also observed by Peron et al. (2008), when evaluating bone marrow cells of Wistar rats treated *in vivo* with the aqueous extract of this plant by gavage at concentrations of 6.85 and 68.50 mg/ml. On the other hand, Pinho et al. (2010), when evaluating the *in vivo* and *in vitro* mutagenicity of the *B. trimera* tea at doses of 20 (usual dose) and 200 g/L (10 times more concentrated), by the *Allium cepa* L. and chromosomal aberration tests in human lymphocytes, highlighted the need for moderation in consumption.

G. cambogia is a plant widely used in the treatment of obesity, and hydroxycitric acid, found in fruit skins, is the substance responsible for the inhibition of lipid synthesis and for the satiating properties of the plant (Santos et al., 2007). In relation to its toxicity, Saito et al. (2005) observed that diets containing *G. cambogia* (hydroxycitric acid at levels higher than 10 g/kg of diet), administered to obese Zucker rats, proved highly toxic for their testicles and caused a decrease in spermatogenesis after 13 weeks of treatment. In the case of Wistar rats, ethanol extracts of *G. cambogia* administered for six weeks showed toxic effects on the reproductive system depending

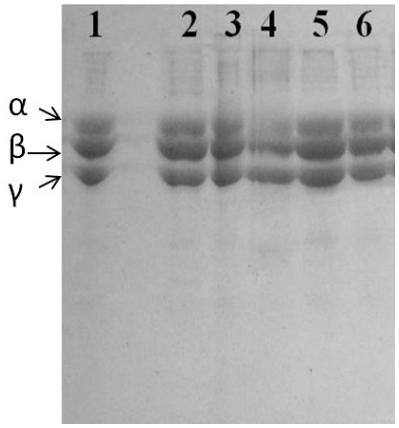

Figure 2. SDS-PAGE for the visualization of the fibrinogenolytic activity. Evaluation of the interaction between fibrinogen and the aqueous extracts of medicinal plants. Samples: 1- Fibrinogen control (80 µg); 2- Fibrinogen (80 µg) + *Garcinia cambogia* (200 µg/ml); 3- Fibrinogen (80 µg) + *Tournefortia paniculata* (200 µg/ml); 4- Fibrinogen (80 µg) + *Baccharis trimera* (200 µg/ml); 5- Fibrinogen (80 µg) + *Simaba ferruginea* (200 µg/ml); 6- Fibrinogen (80 µg) + *Aloe vera* (200 µg/ml); α, β and γ fibrinopeptides.

on the dose, and distortions in the cells of the germinal epithelium were identified (Kayode et al., 2007).

For humans, no side effects were observed for *G. cambogia* extracts administered in the form of tablets containing 1000 mg of hydroxycitric acid per day for 16 weeks (Hayamizu et al., 2003) and 12 weeks of treatment, and there were no changes in serum levels of the sex hormones testosterone, estrone and estradiol (Hayamizu et al., 2008). In the literature consulted, there are no studies related to the toxicity of the plants *Simaba* and *Tournefortia*, which reflects the very recent concern about the possible harmful effects of medicinal plants.

Although some previously cited authors have reported the toxic action of the extracts of *A. vera* (cytotoxicity and mutagenicity), *B. trimera* (mutagenicity) and *G. cambogia* (cytotoxicity), others described an antimutagenic activity and the lack of mutagenicity and genotoxicity, and the latter are in accordance with the observations made in this study, in which the absence of genotoxic potential for the plant extracts on human lymphocytes was observed. These differences are probably due to variations in environmental conditions (climate, soil, season in which the plants were collected, plant age, parts used), as well as sample preparation, dosage used, type of solvent, incubation time and/or form of treatment.

In the analyzed concentrations, none of the plant aqueous extracts induced the lysis of phospholipids and hemolysis (results not shown), with the exception of *B. trimera* (125 and 250 µg/ml), which induced 100% hemolysis in liquid medium (results not shown), possibly due to the presence of interactions of extract constituents with different structures present in erythrocyte membranes, namely, proteins or steroids.

From the metabolites reported in the literature to be present in *B. trimera* leaves, saponins reported by Simão et al. (2014) have hemolytic potential. The ability of saponins to interact with sterols, present in the plasma membrane of erythrocytes, increases the permeability of this membrane, allowing ions and water into the cells, resulting in their rupture and release of hemoglobin (Karabaliev and Kochev, 2003).

Although surfactant and hemolytic properties are striking features of saponins, they are not common to all saponins and, therefore, it is not possible to conclude that they are responsible for the hemolysis observed in this study. Thus, additional studies should be conducted to verify if these saponins are responsible for the hemolytic potential of the aqueous extract from *B. trimera* leaves.

It is suggested that the hemolytic effect in liquid medium observed in this study is related to changes in the molecular and ionic composition of the medium, resulting in the osmotic lysis of erythrocytes, since the hemolytic effect of *B. trimera* was not reproduced in solid medium; in addition, this extract did not induce phospholipid breakdown in the evaluated concentrations.

Regarding coagulant activity, none of the plant extracts was able to induce coagulation (results not shown) at the tested concentrations, thus suggesting that there are no molecules in the composition of the evaluated aqueous extracts capable of cleaving or interacting with components of the coagulation cascade.

The plant extracts evaluated did not show to be proteolytic on fibrinogen molecules or with binding properties to this protein, since the electrophoretic profile of fibrinogen remained unchanged after incubation in the presence of the extracts for a period of 90 min (Figure 2). Fibrinogen is a plasma glycoprotein with symmetrical dimeric structure composed of three pairs of polypeptide chains (alpha, beta and gamma chains) linked together by disulfide bridges, whose main function is related to coagulation and platelet aggregation. The breaking of its chains can lead to changes in the coagulation process, with a consequent induction of bleeding and/or blood clot formation.

The results obtained in this study did not prove the safe use of the evaluated extracts; although they have not been shown to be hemolytic and genotoxic under the evaluated conditions, a broad characterization comprised of *in vitro* and *in vivo* assays is still necessary, thus fulfilling all stages of preclinical and clinical evaluation.

Conclusion

The aqueous extracts of the studied plants did not cause hemolysis (results not shown), with the exception of *B. trimera* (125 and 250 µg/ml), which induced 100% genotoxic,

phospholipase, hemolytic (except *B. trimera*), coagulant and fibrinogenolytic effects at the evaluated concentrations, higher than the ones popularly recommended for human consumption. However, recommending the use of these plants regarding the possible risks and benefits to human health is premature, and additional studies of toxicity, efficacy and safety are necessary.

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Conflict of Interest

The author(s) declare(s) that they have no conflict of interest to disclose.

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