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Evaluation of genotoxic and cytotoxic effects of antileishmanial extract from *Julocroton triqueter* (Euphorbiaceae)

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ABSTRACT. *Julocroton triqueter* extracts have antileishmanial activity; however, the effect on genetic stability has not been studied. We evaluated genotoxic and cell death induction potential (*in vitro* and *in vivo*) of *J. triqueter* var. *triqueter* hydroalcoholic extracts, as well as their antigenotoxic potential *in vivo*. The *in vitro* genotoxic studies were performed using human leukocytes at four different concentrations. For the *in vivo* tests, Swiss mice were treated with 125, 250 or 500 mg/kg of extract injected intraperitoneally. Antigenotoxic effects of the extract were measured before and after cyclophosphamide treatment. An absence of genotoxic effects was observed both *in vitro* and *in vivo*. In the antigenotoxic studies, no significant difference was observed between the treatments and the positive control, indicating that the extracts did not protect against

damage caused by cyclophosphamide. Hydroalcoholic extracts of *J. triqueter* did not provoke DNA damage at concentrations and doses normally used for antileishmanial treatment; however, they reduced apoptotic cell death and induced necrotic cell death.

Key words: Antigenotoxicity; Cell death; Genotoxicity; *Julocroton triqueter*

INTRODUCTION

Understanding the biological effects of medicinal plant extracts and metabolites is an excellent strategy in searching for novel drugs for the treatment of diverse diseases including leishmaniasis. According to the World Health Organization, leishmaniasis is the protozoonosis with the second largest impact on public health, and it is endemic in 88 countries, of which 72 are developing nations (Marín et al., 2009).

Julocroton triqueter (Lam). Didr. var. *triqueter* (Euphorbiaceae), a Neotropical species known as velame, is found from the southern United States to Uruguay. In Brazil, it is distributed throughout Pará, Piauí, Maranhão to Minas Gerais and São Paulo. It is commonly used in powder form as an antiulcerative, antisyphilitic, diuretic, depurative, and stimulant (Pio Corrêa, 1984). Antiinflammatory, antibacterial and antifungal (Lima et al., 2009a) effects as well as leishmanicidal activity against *Leishmania amazonensis* promastigotes have also been reported (Bezerra et al., 2006).

There is currently no information about the effects of extracts of *J. triqueter* (Lam.) Didr. var. *triqueter* on genotoxicity. Therefore, it is necessary to evaluate their effects on DNA, as many plants may possess important pharmacological properties but mutagenic potential at the same time, causing changes in DNA (Marques et al., 2003).

The aim of the current study was to evaluate the genotoxic potential, *in vitro* and *in vivo*, of the hydroalcoholic extract of *J. triqueter* leaves and to evaluate its protective effects against DNA damage induced by cyclophosphamide using two different protocols: pre- and posttreatment. We utilized the comet assay, which is a very useful method for studying genotoxicity and antigenotoxicity *in vivo* and *in vitro*, because it allows genomic lesions to be observed directly (Tice et al., 2000). Additionally, we investigated the extract's ability to induce apoptotic and necrotic cell death in human leukocytes *in vitro*.

MATERIAL AND METHODS

Plant material

J. triqueter leaves were collected from a plant grown in the Berta Langes de Morretes Garden during March 2009, and a voucher specimen was deposited in the Ático Seabra Herbarium under No. 1265. Dried, ground leaves of *J. triqueter* (312 g) were extracted by macerating with ethanol for 72 h, replacing the solvent every 72 h for 30 days. At the end of the process, the extracts were combined and filtered. The total macerate was concentrated in a rotary evaporator at low pressure. This produced 8.58 g hydroalcoholic extract with a 2.75% yield.

Phytochemical analysis

The hydroalcoholic extract of *J. triqueter* was subjected to phytochemical analysis for various substances including anthocyanins, anthocyanidins, flavonols, flavones, flavanonols, xanthones, chalcones, leucoanthocyanidins, catechins, flavanones, resins, phenols, tannins, coumarins, steroids, triterpenes, saponins, alkaloids, and strong nonvolatile acids according to techniques described by Matos (1997).

In vitro assays

Genotoxic studies

Leukocytes from the peripheral blood of 6 volunteers, 3 from each 6, were separated using Ficoll-Paque Plus (GE Healthcare/density: 0.12 EU/mL). The cell suspension (200 µL) was diluted in 5 mL RPMI 1640 culture medium (80%), supplemented with fetal bovine serum (25%), the antibiotics streptomycin and penicillin (1%), and phytohemagglutinin (3%). The cells were immediately treated with 15, 30, 60, or 120 µg/mL hydroalcoholic extract of *J. triqueter*. These concentrations were determined based on the leishmanicidal activity of the extract, with an IC_{50} = 29.5 µg/mL (Bezerra et al., 2006).

Cell viability was measured by adding 10 µL Trypan blue (0.4%) to 10 µL cell suspension. We analyzed 100 cells from each treatment group and determined that the minimum viability of the cells was 70% (Tice et al., 2000).

Cells previously treated with 10 µL 30 mg/mL V10 hydrogen peroxide were used as the positive control. Cells in RPMI 1640 were used as the negative control and dimethyl sulfoxide (1% DMSO) was used as the vehicle control. In all treatments, cells were exposed to the extract for 3 and 24 h (comet assay) and 48 h (cell death assay).

Cell death measurements

Cells were stained with 5 µL of a mixture of fluorescent dyes (25 µL 5 µL/mL propidium iodine, 50 µL 15 µg/mL fluorescein diacetate, 10 µL 2 µL/mL Hoechst 3334, and 15 µL PBS, pH 8) and incubated at 37°C for 5 min. Five hundred cells per culture (3000 per treatment group) were analyzed using a fluorescent microscope (triple filter). The cells were classified as normal (round nuclei stained blue and cytoplasm stained green), necrotic (round nuclei stained red) and apoptotic (nuclei stained blue with apoptotic bodies and green cytoplasm).

In vivo assays

Genotoxicity studies

Thirty male and 30 female Swiss mice (N = 60) at reproductive maturity, approximately 60 days of age, with an average weight of 30 g were provided by the Central Animal House of Maranhão Federal University. The animals were maintained in a polypropylene box during the treatment period (10 animals per group) with water and food *ad libitum* and were maintained at a constant temperature of 26° ± 2°C with a 12-h light/12-h dark cycle. All ex-

perimental procedures were approved by the Ethics and Research Committee of Maranhão Federal University, Protocol No. 23115-012975/2008-43, and performed in accordance with the guidelines of the Brazilian College of Animal Experimentation (COBEA).

Each animal received 0.1 mL of the appropriate treatment by intraperitoneal (*ip*) injection per 10 g body weight. The doses used were 500, 250 and 125 mg/kg, which were estimated using the concentrations that had antileishmanial effects and were limited by their solubility for *ip* treatment. Cyclophosphamide (Sigma, No. 43H0269) (50 mg/kg), sterile water and 1% DMSO were used as the positive, negative and vehicle controls, respectively. The comet assay was carried out with peripheral blood samples collected from each animal 3 and 24 h after administration of the hydroalcoholic extract of *J. triqueter*.

Antigenotoxicity studies

The experimental conditions were similar to those described for the genotoxic studies *in vivo*. A 500 mg/kg dose of hydroalcoholic extract of *J. triqueter* was used for each treatment and administered by *ip* injection. Three types of treatment were tested: 1) pretreatment: extract followed by 50 mg/kg cyclophosphamide after 24 h, 2) post-treatment: 50 mg/kg cyclophosphamide followed by extract after 24 h, and 3) extract control. At 48 h after the initial treatment, peripheral blood cells were collected by tail bleeding.

Comet assay

Two hundred microliters of low melting point agarose (0.5%) was added to 20 µL cell suspension. This mixture was applied to slides pretreated with normal melting point agarose (111194: Pronadisa) (1.5%). The slides were coverslipped and refrigerated for 5 min. The slides were then immersed in ice-cold lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 10% DMSO, 1% Triton X-100, pH 10.0) and refrigerated at 4ºC for 2 h. Next, the slides were incubated for 20 min in an alkaline solution (10 M NaOH, 0.2 M EDTA and distilled water, pH 13), followed by electrophoresis at 25 V and 300 mA for 25 min. The slides were neutralized (0.4 M Tris/HCl, pH 7.5) for 15 min, dried at room temperature and fixed in 100% ethanol. The slides were stained with 20 µg/mL ethidium bromide and analyzed under a fluorescent microscope (BX51/BX52-Olympus; filter 516-560 nm; barrier filter of 590 nm, 40X objective). One hundred nucleoids per treatment were analyzed by considering both the size and the quantity of DNA present in the comet tail. DNA damage was classified into 5 levels: Class 0 - no damage (<5%); Class 1 - low levels of damage (5-20%); Class 2 - moderate levels of damage (20-40%); Class 3 - high levels of damage (40-94%); Class 4 - complete damage (95%). Based on the classes obtained, the score of the damage was calculated by multiplying the number of nuclei in each class by the value of the respective class (Speit and Hartmann, 1995).

Statistical analysis

For the genotoxicity and antigenotoxicity assays, the normality of the data was evaluated by the Shapiro-Wilk test. The *in vitro* and *in vivo* assays were analyzed by the non-parametric Kruskal-Wallis test followed by the Student Newman-Keuls test. The data from the cell death assay were analyzed by the non-parametric chi-square test with a cutoff of $P < 0.05$.

RESULTS

Phytochemical analysis

Phytochemical analysis of *J. triqueter* showed high levels of triterpenes, saponins, alkaloids, and hydrolyzable tannins, modest levels of coumarins and small amounts of flavanones and flavonols. Resins and strong nonvolatile acids were absent.

Cytotoxicity

Cell viability analysis after a 3-h exposure time did not reveal cytotoxicity at the 4 concentrations tested (15, 30, 60, and 120 µg/mL), nor for the controls, with values of 94, 96, 93, 88, 94, and 93%, respectively. Similar results were obtained after an exposure time of 24 h, with viability of 94, 93, 91, 88, 94, and 92%, respectively. The treatments used were therefore within the cytotoxic limits (≥70%) permitted for performing the comet assay.

Genotoxicity studies

In vitro

Treatment with the hydroalcoholic extract of *J. triqueter*, regardless of the concentration, did not result in a significant increase in DNA damage at either time point (3 and 24 h), as classes 0 and 1 were the most frequently observed. Class 0 was the most frequently observed in the negative control, while class 4 was the most common in the positive control.

There were no significant differences in damage scores between the various treatment groups when compared to the negative control or when compared to each other (Table 1).

In vivo

For the doses tested (500, 250 and 125 mg/kg), classes 0 and 1 were the most commonly observed, indicating that the hydroalcoholic extract of *J. triqueter* lacked a genotoxic effect. Class 0 was the most common class for the negative control and class 4 nucleoids were most common in the positive control. When males and females were analyzed separately, a statistical difference was not seen between the genders. There was no significant difference in scores between the groups treated with the extract and the negative and vehicle control groups.

There was also no significant difference when the hydroalcoholic extract of *J. triqueter* treatment groups were compared with each other (Table 2).

Cell death studies

The chi-square test showed a significant difference ($P < 0.0001$) when the frequencies of normal, necrotic and apoptotic cells were compared, indicating that the hydroalcoholic extract of *J. triqueter* altered the proportion of these types of cells at the concentrations tested.

When analyzed separately, the proportion of necrotic cells for the following concentrations was significantly different: 15 µg/mL ($\chi^2 = 127.393$, $P < 0.0001$), 30 µg/mL ($\chi^2 = 220.600$, $P <$

Table 1. Damage scores and frequency of classes detected by the comet assay in leukocytes from peripheral human blood treated with various concentrations of hydroalcoholic extract from *Julocroton triqueter* for 3 and 24 h (N = 6).

Group	Classes (%)					Scores	
	0	1	2	3	4	Median	Midpoint
3 h							
RPMI media	88.0	6.5	4.0	1.0	0.5	0.19	16.17[a]
1% DMSO	86.5	6.2	3.5	1.5	2.3	0.19	17.83[a]
H_2O_2	4.3	2.0	1.7	5.7	86.3	3.94	39.50[b]
JHE (15 µg/mL)	86.3	7.8	3.4	1.8	0.7	0.21	19.42[a]
JHE (30 µg/mL)	86.0	8.7	1.8	1.3	0.7	0.15	16.92[a]
JHE (60 µg/mL)	86.0	10.0	1.6	1.2	1.2	0.15	15.83[a]
JHE (120 µg/mL)	78.8	13.5	3.7	1.8	2.2	0.32	24.83[a]
							H = 17.34
							P = 0.0081
24 h							
RPMI media	85.2	8.3	2.7	1.3	2.5	0.19	17.42[a]
1% DMSO	88.2	6.5	2.0	1.8	1.5	0.09	12.33[a]
H_2O_2	0.5	0.0	0.7	3.3	95.5	3.96	39.50[b]
JHE (15 µg/mL)	80.7	12.1	2.5	2.0	2.7	0.29	19.75[a]
JHE (30 µg/mL)	81.4	12.8	5.0	0.3	0.5	0.23	18.33[a]
JHE (60 µg/mL)	79.8	14.3	4.2	0.3	1.4	0.31	19.75[a]
JHE (120 µg/mL)	74.6	15.7	5.5	2.5	2.0	0.32	23.42[a]
							H = 17.74
							P = 0.0069

JHE = hydroalcoholic extract from *J. triqueter*. Data were analyzed using the Kruskal-Wallis test as they were not normally distributed. Superscript letters denote $P < 0.05$.

Table 2. Damage scores and class frequencies detected by the comet assay in leukocytes from the peripheral blood of Swiss mice treated with various doses of hydroalcoholic extract from *Julocroton triqueter* for 3 and 24 h (10 animals per treatment group).

Group	Classes (%)					Scores	
	0	1	2	3	4	Median	Midpoint
3 h							
Sterile water	86.8	6.6	1.4	0.9	4.3	0.25	29.85[a]
1% DMSO	85.2	8.9	1.1	1.2	3.6	0.21	26.15[a]
Cyclophosphamide	69.0	15.7	3.5	2.2	9.6	0.63	48.40[b]
JHE (125 mg/kg)	81.9	13.9	1.3	0.5	2.4	0.28	28.55[a]
JHE (250 mg/kg)	84.1	10.7	1.6	0.4	3.2	0.16	22.75[a]
JHE (500 mg/kg)	84.6	10.4	1.7	1.5	1.8	0.24	27.30[a]
							H = 13.58
							P = 0.0185
24 h							
Sterile water	90.2	6.8	0.9	0.8	1.3	0.16	18.10[a]
1% DMSO	83.8	10.1	1.5	1.0	3.6	0.32	31.30[a]
Cyclophosphamide	65.8	17.3	3.7	2.6	10.6	0.57	49.30[b]
JHE (125 mg/kg)	83.4	9.3	2.1	1.8	3.4	0.33	31.05[a]
JHE (250 mg/kg)	84.7	10.2	1.5	0.8	2.8	0.30	28.60[a]
JHE (500 mg/kg)	86.8	9.3	1.2	0.6	2.1	0.21	24.65[a]
							H = 17.91
							P = 0.0031

JHE = hydroalcoholic extract from *J. triqueter*. Data were analyzed using the Kruskal-Wallis test as they were not normally distributed. Superscript letters denote $P < 0.05$.

0.0001), 60 µg/mL ($\chi^2 = 141.631$, $P < 0.0001$), and 120 µg/mL ($\chi^2 = 8.814$, $P = 0.0030$). Therefore, we observed that all concentrations tested were able to induce cell death by necrosis. Conversely, there was a statistically significant decrease in cell death by apoptosis ($P < 0.05$) seen between any concentration of hydroalcoholic extract of *J. triqueter* and the negative control (C1: $\chi^2 = 13.24$, $P = 0.0003$; C2: $\chi^2 = 10.34$, $P = 0.0013$; C3: $\chi^2 = 5.22$, $P = 0.0223$; C4: $\chi^2 = 6.35$, $P = 0.0117$) (Table 3).

Table 3. Cell death induction in human leukocytes treated with hydroalcoholic extract from *Julocroton triqueter* for 48 h (N = 6).

Groups	Normal	Cells	
		Necrosis (%)	Apoptosis (%)
RPMI media	2490	437 (15)	73 (2)
1% DMSO	2492	436 (15)	72 (2)
JHE (15 µg/mL)	2172	799 (27)**	29 (1)*
JHE (30 µg/mL)	2042	928 (31)**	30 (1)*
JHE (60 µg/mL)	2142	818 (27)**	40 (1)*
JHE (120 µg/mL)	2430	526 (18)*	44 (1)*

JHE = hydroalcoholic extract from *J. triqueter*. *P < 0.05; **P < 0.0001.

Antigenotoxicity study

There was no statistically significant difference in the damage scores between the negative and vehicle control groups or between the negative and plant extract groups. However, there was a difference between the control groups and the positive control, confirming the validity of the data. Although the damage score was somewhat reduced in both the pre- and post-treatment groups due to cyclophosphamide, the differences were not statistically significant (Table 4). Therefore, the hydroalcoholic extract of *J. triqueter*, at the doses tested, was not able to reduce the genetic damage caused by cyclophosphamide.

Table 4. Damage scores and frequencies of classes detected by the comet assay in leukocytes from the peripheral blood of Swiss mice treated with one dose (500 mg/kg) of hydroalcoholic extract from *Julocroton triqueter* and cyclophosphamide (50 mg/kg) along with their respective controls (10 animals per treatment group).

Group	Class (%)					Scores	
	0	1	2	3	4	Median	Midpoint
Sterile water	90.2	6.8	0.9	0.8	1.3	0.16	11.85[a]
1% DMSO	83.8	10.1	1.5	1.0	3.6	0.32	25.95[a]
Cyclophosphamide	65.8	17.3	3.7	2.6	10.6	0.57	46.10[b]
Plant extract	81.2	13.4	2.2	1.1	2.1	0.30	24.30[a]
Pretreatment	70.6	20.0	5.3	2.3	1.8	0.38	38.40[b]
Posttreatment	74.0	16.6	4.7	2.5	2.2	0.42	36.40[b]
							H = 24.52
							P < 0.0002

Pretreatment = animals treated with extract for 24 h, followed by cyclophosphamide. Posttreatment = animals treated with cyclophosphamide for 24 h, followed by extract. Data were analyzed using the Kruskal-Wallis test as they were not normally distributed. Superscript letters denote P < 0.05.

DISCUSSION

Natural products extracted from plants have important roles in drug discovery; they are used as structural models for the synthesis of novel molecules or exploited for their pharmacological properties. However, studies on the potential toxicological and mutagenic effects of these products to understand the genotoxic effects of new phytotherapeutic agents are still scarce (Vendruscolo et al., 2005). Based on the proven antileishmanial activity of the hydroal-

coholic extract of *J. triqueter* (Bezerra et al., 2006), this study analyzed its genotoxic and antigenotoxic effects, as well as its ability to induce cell death.

We first performed a phytochemical analysis of the plant leaves, which revealed the presence of alkaloids, saponins, hydrolyzable tannins, triterpenes, flavanones, flavanols, and coumarins. These results are similar to findings of previous studies of this genus (Zhang et al., 2008; Lima et al., 2009b).

DNA damage was determined using the comet assay, which has been increasingly used to measure genotoxic potential because it is fast, sensitive and inexpensive. It is widely used to investigate genetic damage associated with exposure to potentially genotoxic agents both *in vitro* and *in vivo* (Lovell and Omori, 2008).

Analysis of the comet assay indicated a lack of genotoxicity for all concentrations of the hydroalcoholic extract of *J. triqueter* tested both *in vivo* and *in vitro*. These results suggest that the extract did not induce DNA damage before or after being subject to cell metabolism.

This study is a pioneering research for this species, and therefore, the literature lacks data on the genotoxic effects of the hydroalcoholic extract of *J. triqueter*. Therefore, our study on the hydroalcoholic extract of *J. triqueter* and DNA damage was compared with studies by other investigators who used other species and/or genera. In the majority of these studies, a significant increase in genetic damage caused by plant extracts was also not seen. Likewise, Maistro et al. (2004) investigated ethanol extracts of *Casearia sylvestris* and also observed that the majority of the cells analyzed had little damage (class 1). Similarly, Santos et al. (2010), who investigated the mutagenicity of chloroform extracts of two species of the genus *Alchornea* (Euphorbiaceae) using the Ames test, did not find evidence of genotoxic effects.

We did, however, observe that the hydroalcoholic extract of *J. triqueter* induced cell death by necrosis *in vitro* at the concentrations tested. A study performed by Bezerra et al. (2006) showed that the hydroalcoholic extract of *J. triqueter* contains 100% of the leishmanicidal activity against promastigotes of *Leishmania amazonensis*, demonstrating a high cytotoxicity against the parasite. Interestingly, the induction of necrosis results in the activation of a cascade of inflammatory reactions, which results in parasite death (Orlofsky et al., 2002). Thus, the hydroalcoholic extract of *J. triqueter*, in addition to being leishmanicidal, also promotes necrotic cell death at the same concentrations at which leishmanicidal activity was shown. Therefore, we suggest that the induction of necrotic cell death may be one of the mechanisms that potentiate the effect of this extract in combating *L. amazonensis*.

The predominance of necrotic cell death may also be explained by one of the characteristics of this type of death. It is known that when a cell dies by necrosis, the plasma membrane ruptures, releasing the cell's contents. This can promote death in neighboring cells, leading to a cascade that results in injury to a large number of cells (Curtin et al., 2002). Among the metabolites encountered in the extract of *J. triqueter*, the saponins are of interest because they are able to lyse cell membranes and cause cell destruction by necrosis (Sparg et al., 2004). Therefore, the predominance of necrotic cell death observed in this study might have also been due to the presence of saponins.

Apoptotic cell death, considered to be a clean and silent death, is fundamentally important for homeostasis and tissue development and has various conserved mechanisms and distinguishing morphological features (Opferman and Korsmeyer, 2003). In this study, we observed a significant decrease in apoptotic cell death. These results are consistent with a lack of genotoxicity, and compounds in the extract have been shown to be able to reduce DNA damage (Magesh et al., 2008). However, other studies performed on species belonging to the family Euphorbiaceae have shown apoptosis induction (Amirghofran et al., 2006; Valadares et al., 2007).

It is known that an agent's capacity to induce genetic damage can be directly related to its metabolism, so the genotoxic effects *in vitro* may be different than those seen in *in vivo* assays (Lima et al., 2010). To test whether components in the extract would have a different effect on DNA after metabolism, the genotoxic potential of the extract was tested in Swiss mice. Our results demonstrated that the three doses tested did not cause DNA damage, similar to the results obtained *in vitro*. The statistical difference between the negative and vehicle controls and the positive control confirms the clastogenic potential of this antitumor compound, validating the data shown in this study.

Previous studies on other plant extracts belonging to the Euphorbiaceae family or on compounds that have a similar phytochemical composition support the results shown here (Oliveira and Nepomuceno, 2004; Strange et al., 2009). Munari et al. (2010) proposed that the lack of genotoxic effects *in vivo* may indicate that the compounds present in a plant extract are biotransformed in the liver, leading to their inactivation. However, we found a lack of genotoxic effects both *in vitro* and *in vivo*.

Based on our antigenotoxicity results, we show that the extract does not have a significant protective effect against genotoxic damage caused by cyclophosphamide, although we cannot rule out the possibility that this effect may be seen at higher doses. Carvalho et al. (2011) reported that the antigenotoxic activity of the extract may be directly dependent on the dose that is used. In the present study, we observed a trend towards damage reduction in the groups treated with the plant extract.

The lack of genotoxicity or potential antigenotoxic activity in the hydroalcoholic extract of *J. triqueter* may be due to the presence of metabolites found in this extract, especially flavonoids, of the flavanol and flavanone classes. The biological activity of these compounds has been attributed to their antioxidant effects, especially their ability to neutralize reactive oxygen species, alleviating the negative effects of these species on cells (Williams et al., 2004). Conversely, some flavonoids have been shown to be mutagenic and genotoxic in prokaryotic and eukaryotic cells (Skibola and Smith, 2000). The mechanism that describes the mutagenic and genotoxic activity of the flavonoid depends on its structure and/or its metabolism or production of reactive oxygen species such as superoxides and hydroxyls (Silva et al., 2000).

Hydrolyzable tannins, which have antioxidant activity, are found in the hydroalcoholic extract of *J. triqueter* (Monteiro et al., 2005). In addition, they may also have antimutagenic and antitumor effects (Dauer et al., 2003). However, these polyphenols, similar to the flavonoids, have pro-oxidant effects in the presence of metal ions and can thereby act as cytotoxins and genotoxins (Labieniec et al., 2003).

We also showed the presence of saponins in the hydroalcoholic extract from *J. triqueter*. Tests performed with saponins *in vitro* have investigated their mutagenic and antimutagenic effects. These compounds did not induce the formation of micronuclei in human lymphocytes and inhibited the cytotoxic and genotoxic effects induced by mitomycin C, bleomycin, and other drugs (Scarpato et al., 1998).

Several studies have investigated the mutagenic and genotoxic potential of coumarin, another metabolite found in the hydroalcoholic extract from *J. triqueter*. In general, the data suggest that coumarin is not a genotoxic agent (Lake, 1999). Api (2001) also showed that coumarins do not have mutagenic effects when studied *in vivo* by the micronucleus test.

We also reported the presence of triterpenes in the extract of *J. triqueter*. Studies performed by Villaseñor et al. (2004) attributed the lack of mutagenicity in the extracts that they studied to the presence of flavonoids and triterpenes. Magesh et al. (2008) have also reported an antimutagenic

activity by this metabolite, supporting the results obtained in the present study on *J. triqueter*.

Many alkaloids are recognized as having genotoxic and mutagenic effects as well as antimicrobial and antiplasmodial activity (Ansah et al., 2005; Mei et al., 2005). However, alkaloids do not exhibit mutagenic effects in the Ames test and do not induce micronuclei in Swiss-Webster mice (Friedman and Henika, 1992). Reported studies have demonstrated the antioxidant effects of alkaloids as they are especially efficient against hydroxyls (Fragoso et al., 2008).

Overall, the results obtained in this study showed that, both *in vivo* and *in vitro*, extracts of *J. triqueter* leaves, rich in secondary metabolites lack a genotoxic effect. The phytochemical analysis showed that the majority of the substances present have DNA-protective and not DNA-damaging properties.

In light of these results, the hydroalcoholic extract from *J. triqueter* (Lam). Didr. var. *triqueter* is a promising phytotherapeutic product for treating leishmaniasis, as it does not show genotoxic effects *in vitro* or *in vivo*, contrary to Glucantime®, the drug currently used to treat this disease, which has a proven mutagenic effect (Lima et al., 2010). In addition, this hydroalcoholic extract is capable of reducing apoptotic cell death and inducing necrotic cell death at anti-leishmanial concentrations.

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