

ABSENCE OF MUTAGENICITY OF PLANTS USED TO TREAT GASTROINTESTINAL DISORDERS

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Abstract - The Brazilian Savanna (locally called “Cerrado”) is an important biome presenting several plants that are used in popular medicine. However, the risks associated with the consumption of derivatives from these plants are generally unknown. Studies with compounds obtained from different species have shown the risks of DNA damage. The present work assessed the *in vivo* mutagenicity of three plant species used in popular medicine to treat human gastrointestinal disorders (*Mouriri pusa*, *Qualea grandiflora* and *Qualea multiflora*). The micronucleus assay was performed in peripheral blood of mice submitted to acute treatments. Results showed that no assessed extracts were mutagenic *in vivo*. In fact, the absence of mutagenicity in the present study indicates that the extracts do not contain compounds capable of inducing DNA breaks or chromosomal loss. However, further analysis should be performed in others systems to guarantee their safety, mainly to human chronic use.

Key words: Mutagenicity, micronucleus assay, medicinal plants, Brazilian savanna, DNA damage.

INTRODUCTION

According to the World Health Organization (WHO), about 80% of the world population uses medicinal plants or practice treatments suggested by traditional medicine (Basso et al., 2005). Several allopathic medicaments produced nowadays and consumed on a large scale are derived from natural products. About 25% of the medicaments are prescribed worldwide and 11% of the medicaments are considered essential by the WHO are products derived from plants (Rates, 2001).

However, the risks associated with the consumption of natural products must be considered. It is important to note that natural plant extracts constitute

complex mixtures of a great number of chemical substances, mainly organic compounds, and that some of these compounds may exhibit significant toxicity. Furthermore, the concentrations of each of these compounds can vary pronouncedly, depending on the region, culture, weather, and others factors. Indeed, different works have shown that several plants used in medical practice may contain substances that are toxic to organisms, inducing, for instance, DNA damage (Akintonwa et al., 2009; Stanic et al., 2009; Verschaeve and Van Staden, 2008). According to Ames et al. (1983), plants utilized in diet and employed in medical treatments possess chemical compounds that are capable of inducing mutations. Studies performed recently have demonstrated that

extracts and enriched fractions obtained from Brazilian medicinal plants were mutagenic *in vitro* and *in vivo* (Santos et al., 2006a; Santos et al., 2008b).

The Brazilian Savanna (locally called “Cerrado”) is an important biome presenting approximately 7000 vegetal species. Studies carried out with the natural products obtained from the plants of this biome have demonstrated important biological activities in different systems (Lira et al., 2008; Mazzolin et al., 2010). However, the inherent risks associated with the use of medicinal plants should not be overlooked. In the present study we examined the mutagenicity produced by three vegetable species from Cerrado used in popular medicine to treat gastrointestinal disorders, such as diarrhea with blood and intestinal colic (Gaspi et al., 2006; Pott and Pott, 1994). This was evaluated *in vivo* using the micronucleus assay of peripheral blood cells of mice submitted to acute treatment.

MATERIALS AND METHODS

Species evaluated and extract obtaining

In the present study, three vegetal species from Cerrado were evaluated: *Mouriri pusa* Gardner (Melastomataceae), *Qualea grandiflora* Mart. (Vochysiaceae) and *Qualea multiflora* Mart. (Vochysiaceae).

Leaves of *M. pusa* were collected in the district of Brejinho de Nazaré (Tocantins State, Brazil). A voucher specimen was kept in the University of Tocantins (UNITINS) herbarium (No. 4548). The bark of *Q. grandiflora* and *Q. multiflora* were collected at Pedro Afonso Road (Tocantins State, Brazil) and identified by Solange de Fatima Lolis (UNITINS). The voucher specimens were kept in the University of Tocantins herbarium (No. 3379 and No. 4158, respectively).

The leaves of *M. pusa* and bark of *Q. grandiflora* and *Q. multiflora* were air-dried and powdered. Individually, these vegetal materials were exhaustively extracted with methanol (48 h) at room temperature. The solvent was evaporated at 60°C, under reduced

pressure, in order to obtain the four different methanol extracts evaluated in this study. The yield of the extraction process is presented in Table 1.

Micronucleus assay

To perform the micronucleus assay, blood samples from five to six week-old albino Swiss mice (*Mus musculus*), weighing approximately 30 g, from the Central Animal Facility of the State University of Londrina (Parana – Brazil) were used. The animals were kept individually in polypropylene cages following the conditions for animal care recommended by the Canadian Council on Animal Care (Olfert et al., 1993). All the procedures were approved by the Ethics in Research Committee of the São Paulo State University in Araraquara (UNESP, SP, Brazil).

The mice were divided into eleven groups of ten animals (5 males and 5 females) for the evaluation of the different vegetable extracts: a negative control group, which receive distilled water, a positive control group, treated with cyclophosphamide (40 mg Kg⁻¹ body weight (b.w.)) and nine groups that received the vegetal extracts. Each extract was assessed in three different doses: *M. pusa* – 665.0, 997.5 and 1330 mg Kg⁻¹ b.w.; *Q. grandiflora* – 909.0, 1363.0 and 1818 mg Kg⁻¹ b.w.; *Q. multiflora* – 855.8, 1283.7 and 1711.6 mg Kg⁻¹ b.w. These doses were based in the solubility limit of the extracts in distilled water, being that the dosages tested in the present work were not toxic to the animals. Each animal in these groups was treated with 0.1 mL of each test solution per 10 g body weight. The animals received water and food *ad libitum* between the treatment and the blood sampling. The treatments with plant extracts and with distilled water (negative control) were performed via gavage. The treatment with cyclophosphamide was performed intraperitoneally.

The micronucleus test on peripheral blood cells was conducted as described by Hayashi et al. (Hayashi et al., 1990). The animals received single doses of test solution (controls and vegetable extracts) 30 h before the collection of blood samples. Blood was collected by perforating the caudal vein of the mouse

with a needle and collecting 5 μ L drops, each of which was placed at the center of a pre-stained slide and covered with a coverslip (24 x 40 mm).

The cell preparations were examined under a fluorescence microscope, with a blue (488 nm) excitation filter and yellow (515 nm) emission (barrier) filter, using an oil-immersion objective. One thousand reticulocytes per treated animal were analyzed and the proportion of micronucleated cells was counted. For the statistical analysis, mean and standard deviations were calculated and ANOVA was performed. The Tukey-Kramer test was employed to compare the results obtained for the treated groups with those of the negative control group.

RESULTS AND DISCUSSION

The secondary metabolites of plants may have biological activity variables, such as antibiotic, pesticide, repellent, controllers of cell division, among others (Mitscher et al., 1996). In this way, these activities can represent biological risks to organisms exposed to compounds obtained from vegetal sources.

In fact, Ames (Ames, 1983) pointed out that many elements found in the human diet could have noxious effects. Other studies have demonstrated that natural products can represent serious risks to the DNA integrity of different organisms (Calvo et al., 2011; Santos et al., 2010).

Such information underlines the importance of studying the genetic risks of plant compounds, especially in plants utilized by humans in the diet or in medicinal treatments. In the present study, we evaluated the mutagenicity of four vegetal species from the Brazilian Cerrado, employing the micronucleus assay in peripheral blood of mice submitted to acute treatments. All the species analyzed are commonly applied in popular medicine to treat several diseases, especially those related to gastrointestinal disorders.

Data presented in Table 2 show that none of plant species assessed in this study was mutagenic in the micronucleus assay. All evaluated extracts were

unable to induce DNA damage in mice submitted to acute treatment with different doses of these vegetal compounds.

In *M. pusa*, as observed in our previous work (Santos et al., 2008b), the phenolic compound quercetin was responsible for the induction of DNA damage in *Salmonella typhimurium* assay. However, herein we observed that the methanol extract of *M. pusa* was not mutagenic *in vivo*. This can be explained by the fact that the micronucleus assay identifies agents that cause DNA breaks or loss of whole chromosomes as the *Salmonella typhimurium* assay identifies agents that cause point mutations. Besides, according to Silva et al. (2002), quercetin aglycone, when evaluated at high doses, presents lower mutagenicity *in vivo* compared to *in vitro* studies. This compound presents rapid degradation and elimination processes *in vivo*, which corroborates the explanation of differences observed between *in vivo* and *in vitro* results.

The *Q. grandiflora* and *Q. multiflora* extracts, as discussed above, were not mutagenic *in vivo*. Phytochemical studies with this vegetal species showed the presence of terpenes and saponins and, in agreement with the literature, the majority of these compounds are not mutagenic (Agner et al., 1999; Berhow et al., 2000). In addition, studies have shown that some terpenes and plant extracts with these compounds in their phytochemical composition can present anti-mutagenicity and antiapoptotic activities (Nikolic et al., 2011; Santos et al., 2006b; Santos et al., 2008a).

The use of plants with mutagenic potential in medical treatments can represent serious risks to patients since the mutations are closely related to the development of diseases such as cancer (De Flora, 1998). The methodology employed herein permitted the identification of agents that are capable of inducing DNA breakage (clastogenesis) or mitotic fails that result in chromosome loss (aneuploidy). The presented data show that the species evaluated in this study did not induce mutagenic effects in mice. However, these results cannot be considered a safety indicator for human consumption and further analy-

Table 1. Yields of different plant species obtained after the extraction process.

Species	Methanol Extract Yield
<i>M. pusa</i>	10.02%
<i>Q. grandiflora</i>	10.86%
<i>Q. multiflora</i>	10.22%

Table 2. Micronucleated reticulocytes per animal, mean and standard deviation (SD) in groups of animals treated with different doses of the methanol extracts from vegetal species belonging to Brazilian Cerrado.

Treatments (mg/kg b.w.)	Animals										Mean	SD
	1	2	3	4	5	6	7	8	9	10		
Ctrol +	22	ND	17	18	20	18	18	22	20	19	19.33**	1.80
Ctrol -	2	1	1	3	1	2	1	2	1	0	1.40	0.84
<i>Mouriri Pusa</i>												
1330	0	2	3	2	1	ND	3	1	0	1	1.44	1.13
997.5	2	1	1	2	1	1	0	ND	1	3	1.33	0.87
665	1	2	3	1	2	1	2	1	ND	2	1.67	0.71
<i>Qualea grandiflora</i>												
1818	1	0	2	1	2	1	2	2	1	2	1.38	0.74
1363	1	3	1	2	2	1	3	1	2	1	1.75	0.89
909	1	2	2	2	1	1	3	ND	1	2	1.67	0.71
<i>Qualea multiflora</i>												
1711.6	0	4	1	3	2	2	0	3	3	3	2.10	1.37
1283.7	2	4	4	2	3	2	3	2	2	4	2.80	0.92
855.8	1	2	2	1	3	2	1	2	1	2	1.70	0.67

b.w.: Body weight; Ctrol+: Positive Control – Cyclophosphamide 40 mg Kg⁻¹ b.w.; Ctrol-: Negative Control – distilled water; ND: not determined; SD: Standard Deviation ** P < 0.01.

sis should be performed in other biological systems to guarantee their safety, especially chronic use of the extracts.

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