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Assessment of mutagenic, antimutagenic and genotoxicity effects of *Mimosa tenuiflora*

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Article

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Abstract: Genotoxic effects of *Mimosa tenuiflora* (Willd.) Poir, Fabaceae, were investigated by using both micronucleus test and bacterial reverse mutation assay in *Salmonella typhimurium* TA97, TA98, TA100, TA102 respectively. In respect of Ames test results show that the extract does not induce mutations in any strains of *Salmonella typhimurium* tested since the mutagenicity index is less than 2. In the antimutagenic effect was observed that the extract at the concentrations tested significantly decreased the mutagenicity index of all strains tested which characterized the extract as antimutagenic in these conditions. In the micronucleus test *in vivo*, we observed that the concentrations used did not induce an increase in the frequency of micronucleus in normochromatic erythrocytes of mice. Therefore, we concluded that the extract of *M. tenuiflora* is not mutagenic in the absence of exogenous metabolizing system and does not induce an increase in the frequency of the micronucleus characterized as an agent not mutagenic in these conditions. Further studies of toxicity need to be made to the use of this plant in the treatment of diseases to be stimulated.

Keywords:
Genetic toxicology
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Introduction

The healing power of plants is as old as the emergence of the human species on earth. Early, the first civilizations noticed that some plants contain in their essence, the active ingredients which are tested in combating disease empirically proved its healing power (Badke et al., 2011). On this basis, it is very important to carry out toxicity studies on plant-based extracts, as some of its biologically active constituents may be toxic to the body and contain chemicals known to be mutagenic or carcinogenic.

The lack of adequate information on the medicinal properties of plants (mainly exotic), its concomitant use with traditional medicines (allopathic) without notice to the doctor, and finally the loss of knowledge about the effects of medicinal and toxic plants, as well as the ability to identify them by the migration of rural population to cities are worrying factors in the use of medicinal plants without previous studies (Veiga Júnior et al., 2005; Albuquerque & Hanazaki, 2006).

Genetic toxicology tests are assays designed to detect direct or indirect genetic damage induced by chemical compounds. Fixation of DNA damage can result in gene mutations, loss of heterozygosity, chromosome loss or gain, and chromosome aberrations. These events may play an important role in many malignancies. Thus, identifying genotoxic/mutagenic effects is important for the risk/benefit assessment of substances, in particular those which are part of the dietary habits of any populations (Doppalapudi et al., 2007).

Examining the mutagenicity of plant extract or chemical(s), Ames test, or the so-called *Salmonella*/microsome test, is widely used (Konuk et al., 2008; Uysal et al., 2010; Liman et al., 2010). This test can be carried out rapidly and cheaply and it is also one of the most reliable short-term bacterial test systems (Maron & Ames, 1983; Mortelmans & Zeiger, 2000). A compound is considered mutagenic when the increase in dose is directly related to the increase in number of colonies mutated in one or more strains of *Salmonella typhimurium* dependent histidine (Mortelmans & Zeiger, 2000).

Despite the valuable contribution to the control of many diseases, the use of drugs in medical practice produces undesirable effects, including carcinogenesis. The micronucleus test *in vivo* is widely accepted by international agencies and government institutions as part of the recommended battery of tests to establish the evaluation and registration of new chemicals and pharmaceutical annually entering the world market and

that may have mutagenic activity (Ribeiro & Marques, 2003)

Mimosa tenuiflora (Willd.) Poir., Fabaceae, is a perennial evergreen tree or shrub native of Northeast Brazil where is popularly known as "jurema-preta". It is used in folk medicine against bronchitis, coughs, fever, headache and external ulcers, and in Indigenous and Afro-Brazilian cults as entheogen (Albuquerque et al., 2007; Agra et al., 2008; Souza et al, 2008). Phytochemical investigations of *M. tenuiflora* resulted in the isolation of several classes of compounds, including indole alkaloids, tannins, chalcones, steroids, terpenoids and phenoxychomones (Rivera-Arce et al., 2007; Souza et al., 2008).

Considering the absence of studies on the toxic effects of this plant, the aim of the present study was to evaluate the mutagenic /antimutagenic and genotoxic activities *M. tenuiflora* using the Ames test and the micronucleus test on peripheral blood cells.

Materials and Methods

Chemicals

Ethanol, methanol, D-glucose-6-phosphate disodium salt, magnesium chloride, L-histidine monohydrate, D-biotin, sodium and 4-nitroquinoline 1-oxide were purchased from Sigma Chemical Co (St. Louis,USA). Nutrient Broth medium (Difco/USA) was used as bacterial media. Agar (Difco/USA) was used in the Ames test.

Plant materials

The steam bark of *Mimosa tenuiflora* (Willd.) Poir, Fabaceae, was collected in March 2010 at the Center for Rural Health and Technology, Patos Campus UFCG-Paraíba, Brazil and identified by Denise Aline Cassimiro Bezerra (UFPB). The voucher specimen (#3274) of the plant was deposited in the Herbarium Caririensis Dardanus de Andrade Lima, the Cariri Regional University - URCA, Crato-CE, Brazil.

Animals treatment

The use of animals was approved by the Ethics Committee for Animal Research Laboratory of Pharmaceutical Technology UFPB under registration number 0206/11. For the realization of experimental models were used five to six-week old albino Swiss mice (*Mus musculus*), weighing approximately 30 g from the Biotery Prof. Thomas George UFPB. The animals were acclimated to the bioterium local conditions for about seven days before the experimental tests under temperature (21±2 °C) and controlled light-dark cycle of 12 h. The animals were fed chow and water ad libitum and were distributed in the different experimental groups at random.

Preparation of crude ethanolic extract of Mimosa tenuiflora

The steam bark of *M. tenuiflora* were dried in an oven with circulating air average temperature of 45 ºC for 3 to 4 days, and then subjected to a process of spraying in mechanical mill, reducing it to powder. The dried and powdered plant material, weighing 430 g, was subjected to exhaustive maceration with 2 L of EtOH at 95% for 72 h, this process being repeated three times to obtain maximum extraction of chemical constituents. The resulting extraction solution, after filtration, was concentrated with the aid of a rotary evaporator under reduced pressure at a temperature not exceeding 40 ºC, resulting in a 22% income.

Phytochemical screening of extract of Mimosa tenuiflora

Initially, the extract was analyzed by phytochemical screening for the identification of several classes of secondary metabolites. It was subsequently analyzed by thin layer chromatography for the detection of phenols. Chromate aluminum silica gel plates F 254 (Merck®, Darmstadt, Germany) were used. Flavonoids were analyzed using ethyl acetate:formic acid:water (8:1:1, v/v) as mobile phase natural and reagent A (1%) and UV 365 nm as developer. For tannins, were used as mobile phase ethyl acetate:formic acid:water (9:0,5, 0.5 v/v) and toluene:acetone:acetic acid (6:2:2, v/v) , ferric chloride (5%) for visualization of the spots. We used chromatographic standards of tannins gallic acid, ellagic acid, tannic acid, catechin and epicatechin and flavonoids isoquercetina, isovitexin, quercetin, rutin and vitexin (Extrasynthese and Sigma) (Wagner & Bladt, 1996).

For phytochemical analysis, it was used the methodology proposed by Matos (1995) which consists of a phytochemical semi-quantitative, based on color and precipitation reactions specific for each class of constituents being analyzed. The crosses classification proposed by the same author is the intensity in rate of reaction and correlating the amount of constituents present, and thus a semi-qualitative estimation. Example: For detection of the reaction of tannin was used the technique of precipitation of gelatin. The presence of light (+), moderate (+ +) or severe (+ + +) tannin is represented fairly according to the intensity of these reactions (formation of precipitate).

Ames mutagenic/antimutagenic test

The mutagenicity was evaluated using part of the methodology described by Maron & Ames (1983). Selection of the strains was based on the testing and strain selection strategies of Mortelmans & Zeiger (2000).

Suspensions in Nutrient Broth medium (DIFCO)

of mutant strains of *S. typhimurium* TA97, TA98, TA100, TA102, in stationary phase, which carry different mutations in various genes of the histidine operon (*His*-) were incubated with concentrations of 100 and 50 µg.mL^{-1} of extract of *M. tenuiflora* at a temperature of 37 °C in the absence of exogenous metabolizing system S9 (-S9). These concentrations were used (below the minimum inhibitory concentration) because the extract showed antibacterial activity against *Salmonella* strains tested with MIC of 250 µg.mL^{-1}.

After 30 min, the samples were plated on minimal agar medium, 20 mL Vogel-Bonner medium (10 g $MgSO_4.H_20$, 100 g citric acid monohydrate, 500 g K_2HPO_4, 175 g $Na_2NH_2PO_4.4H_2O$ to 1 L), 50 mL glucose 10% v/v, 15 g agar to 1 L) and incubated at 37 °C for 48 h. Mutant colonies (*His*+) that restored its capacity to synthesize histidine (revertants) were counted. Suspensions were plated *S. typhimurium* as a negative control and suspensions of *S. typhimurium* incubated in the presence of 4-nitroquinoline 1-oxide (NQNO) (0.5 µg/plate) (Sigma), as positive control.

To investigate whether the extract of *M. tenuiflora* has antimutagenic activity, suspensions of *S. typhimurium* (TA97, TA98, TA100, TA102) were subjected to two concentrations of the product (100 and 50 µg.mL^{-1}) at 37 °C for 30 min. Then the samples were incubated in the presence of 4-nitroquinoline 1-oxide for 30 min, plated on minimal agar medium and maintained at 37 °C for 48 h.

All experiments were performed in triplicate and results expressed as mutagenicity index. The mutagenicity index (MI) was also calculated for each dose. MI is the average number of revertants per plate divided by the average number of revertants per plate from the negative (solvent) control. A sample was considered positive when the MI≥2 for at least one of the tested doses and if it gave a reproducible dose-response curve (Varella et al., 2004; Santos et al., 2008). Extracts that presented concentrations that induces significant difference in comparison to negative control revertants frequency and MI higher than 1.5 and lower than 2 were considered as a weak mutagen (Mortelmans & Zeiger, 2000).

Micronucleus test

To perform the micronucleus test, the animals were sacrificed with xylazine (5 mg/kg) in accordance with existing regulations to prevent anxiety or fear (stress) (Andrade et al., 2006) and then blood samples were collected from the caudal vein of mices.

The micronucleus test on peripheral blood cells was carried out as described by Hayashi et al. (1994), who concluded that bone marrow cells can be replaced by peripheral blood as material for the micronucleus assay. This is allowed because, alternatively in mice, the micronuclei can be analyzed in circulating normochromatic erythrocytes (NCE, erythrocytes), whereas the spleen of mice did not hijack the blood micronucleated erythrocytes.

Groups of three mices males and three females received, by gavage, the crude extract of *M. tenuiflora* in dose of 100 to 200 mg/kg of animal weight because according to Pereira (2010) the extract of *M. tenuiflora* has LD50 500 mg/kg. The negative control group received only the dispersant of the sample (distilled water) and positive control received Cyclophosphamide 50 mg/kg of animal weight. Twenty-four hours after treatment, the animals were sacrificed, blood was collected from the caudal vein and made a smear on the slide.

Analysis of the slides

The slides were stained with Panotic and observed under an optical microscope (Zeiss) increasing 1000x (objective 100x with eyepiece 10x) for counting the micronucleus. Were assessed at least 2,000 NCE per slides (Hayashi et al., 1994).

In this study, the presence of micronucleus in erythrocytes of mice in the positive control was not influenced by gender (p>0,05), so data were pooled to determine the average number of micronucleus to calculate the standard error of the mean and to assess differences between groups.

The data from the micronucleus assay were statistically analyzed using Student's t-test, comparing the treated groups with controls (Pereira, 1991). The significance level considered was $p<0.05$. Results were expressed as mean±SEM.

Results and Discussion

Living organisms are often exposed to mutagenic substances, and many of them may have a natural origin, such as example of herbal medicines. Since many extracts and active principles of plants already described have been used as therapeutic agents for considerable interest in determining the risk they may cause health, leading to diseases or even death in animals and humans. Thus, evaluation of mutagenic and/or genotoxic potential were required to ensure the use of relatively safe plants man medicinal as is the case of *Mimosa tenuiflora* (Willd.) Poir, Fabaceae, (Surh & Ferguson, 2003).

Through phytochemical prospecting of the extracts, it was possible to determine the presence of diverse classes of secondary metabolites. The results revealed a wide variety of chemical constituents highlighting the presence of saponins, gums, resins, and particularly phenols (among the flavonoids and tannins) supporting the studies pharmacochemical of Camargo-Ricalde (2000) and Bezerra (2008). The extract of *M. tenuiflora* also showed moderate presence of alkaloids, flavonoids and

pyrogallic tannins; thioglycosides traits were observed and the absence of mucilage, quinones, coumarins, lactones, steroid, carotenoids, catechins and resins. These results corroborate the studies of Meckes-Lozoya et al. (1990a).

Some studies confirm the presence of saponin in the stem of *M. tenuiflora.* Antón et al. (1993) have isolated and identified three new triterpenoid saponins and three steroidal saponins. Total saponin concentration in the whole extract of M. tenuiflora was *ca.* 0.15% (being 0.10% for mimonoside A; 0.032% for mimonoside B; and 0.016% for mimonoside C). The three steroidal saponins were 3-*O*-β-D-glucopyranosyl campesterol, 3-*O*-β-D-glucopyranosyl stigmasterol and 3-*O*-β-D-glucopyranosyl-β-sitosterol) of the stem of *M. tenuiflora.*

Meckes-Lozoya et al. (1990b) also claim that the abundance of tannins and flavonoids found in the extract of "jurema-preta" factor is probably responsible for the observed antimicrobial activity in *Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa* and *Acinetobacter calcoaceticus.*

The Ames test is widely used as a screening method to determine the mutagenic potential of new chemical compounds and drugs since there is a high correlation between positive response in test genetic toxicity and carcinogenicity, both in rodents and in humans (McCann et al., 1975; Purchase et al., 1978; Mortelmans & Zeiger, 2000).

The crude extract of *M. tenuiflora* in the concentration of 100 and 50 μg.mL^{-1} did not induce mutations in strains of *S. typhimurium* tested since the mutagenicity ratio is less than 2 (Table 1), so the extract of *M. tenuiflora* is not considered mutagenic in these conditions evaluated.

The treatment of strains of *S. typhimurium* with crude ethanol extract of *M. tenuiflor*a before incubation with the mutagenic agent (4-nitroquinoline 1-oxide) showed an antimutagenic effect on strains TA97, TA100 and TA102 since the mutagenicity ratio is less than 2 (Table 2). In strain TA98, even the mutagenicity index was greater than 2, it is observed that there was a large reduction of mutagenicity index what characterizes an antimutagenic effect.

The antimutagenic activity of the extract of *M. tenuiflora* is certainly related to the abundance of tannins present in this species (Meckes-Lozoya et al., 1990a) that, according to Dauer et al (2003), has antimutagenic activity.

The assessment of micronucleus induction is the primary *in viv*o test in a battery of genotoxicity tests and is recommended by law enforcement agencies worldwide as part of the safety assessment of chemicals and natural. The test, when performed correctly, detects both effects: clastogenic and aneugenic (Krishna & Hayashi, 2000).

According Cammerer et al (2007) the micronucleus assay done in peripheral blood has further advantages, such as the easy preparation of the sample small amount of blood, the speed in obtaining results, the ability to obtain repeated samples of the same animal, and the possibility of obtaining samples studies chronic.

Micronucleus in young erythrocytes emerge primarily from acentric fragments or chromosomes that are unable to migrate following the mitotic spindle during cell division of hematopoietic tissue (Salamone & Heddle, 1983; Ouanes et al., 2003). An increase in frequency micronucleus test animals treated with different substances is an indication of chromosomal damage induced (Krishna & Hayashi, 2000). Micronuclei are indicative of numerical and/or structural chromosome aberrations during cell mitosis. Other authors have used the micronucleus test as a biomarker for chromosome instability and malignancy, observing higher frequencies of micronucleated cells among cancer patients than among healthy individuals (Kamboj & Mahajan, 2007; Lou et al., 2007).

The results showed that both at the dose of 100 to 200 mg/kg of the extract of *M. tenuiflora* did not induce a significant increase in the numbers of micronucleus in relation to the negative control (p>0,05) not presenting, therefore, clastogenic and aneugenic effect. Only cyclophosphamide (positive control) induced a significant increase in the amount of micronucleus (p<0,05) (Table 3).

Della Torre (2011) investigated the mutagenicity of the extract of *Plathymenia reticulata* Benth (Leguminosae) against strains of *S. typhimurium* TA100,

Table 1. Mutagenic effect of the extract of *Mimosa tenuiflora* on strains of *S. typhymurium.* The results are expressed as mutagenicity index and number of revertants colonies per plate (mean±standard error of mean).

Experimental group	MI (TA 97)	MI (TA 98)	MI (TA100)	MI (TA 102)
NQNO[a] (positive control)	2.37	33.09	13.07	8.98
Revertants/plates (UFC)	316±3,5	320±4,7	932±2,4	348±2,3
Extract 100 μg.mL^{-1}	0.42	0.26	0.41	0.42
Revertants/plate (UFC)	93±2,6	7±2,1	21±5,7	30±3.2
Extract 50 μg.mL^{-1}	0.36	0.73	0.37	0.35
Revertants/plate(UFC)	80±4,6	19±3,2	19±2,5	25±2,6

Values in brackets (MI) ≥2 indicate mutagenicity. NQNO- 4-nitroquinoline 1-oxide.

Table 2. Antimutagenic effect of the extract of *Mimosa tenuiflora* on strains of *S. typhymurium*. The results are expressed as mutagenicity index and number of revertants per plates.

Experimental group	MI (TA 97)	MI (TA 98)	MI (TA 100)	MI (TA 102)
NQNO[a]	2.37	33.09	13.07	8.98
Extract 100 µg.mL^{-1}+NQNO	0	5.3	0.19	0
Extract 50 µg.mL^{-1}+NQN	0	4.4	0.14	0.01

Values in brackets (MI) ≥2 indicate mutagenicity. NQNO- 4-nitroquinoline 1-oxide.

TA98, TA97a, and TA102. Species of this family are known to have high levels of flavonoids and tannins in its constitution. The extract was mutagenic for strain TA98 and showed signs of mutagenicity for TA102 and TA97.

Through the results obtained it can be concluded that the extract of *M. tenuiflora* is not mutagenic in the absence of exogenous metabolizing system and does not induce an increase in the frequency of the micronucleus characterized as an agent not mutagenic in these conditions. Further studies of toxicity need to be made to the use of this plant in the treatment of diseases to be stimulated.

Table 3. Micronucleus frequency in 2000 found peripheral blood erythrocytes of mice of different experimental groups.

Experimental group	Number of micronucleated erythrocytes (mean±SEM)
Control (water)	12.0±2.64
Cyclophosphamide (50 mg/kg)	43.5±5.89**
M. tenuiflora (100 mg/kg)	8.75±2.12
M. tenuiflora (200 mg/kg)	9.91±1.06

Authors' contributions

VAS (PhD student) contributed in collecting plant sample and identification, confection of herbarium, running the laboratory work, analysis of the data and drafted the paper. GFG, AFRF and MFFMD contributed to biological studies. MSVP contributed in plant identification and herbarium confection. IFG contributed to chromatographic analysis. MFFMD and HLFP contributed to critical reading of the manuscript. MSVP contributed to plant collection. HLFP designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

References

Agra MF, Silva KN, Basílio IJLD, Freitas PF, Barbosa-Filho JM 2008. Survey of medicinal plants used in the region Northeast of Brazil. *Rev Bras Farmacogn 18*: 472-508.

Albuquerque UP, Medeiros PM, Almeida ALS, Monteiro JM, Lins-Neto EMF, Melo JG, Santos JP 2007. Medicinal plants of the caatinga (semi-arid) vegetation of NE Brazil: A quantitative approach. *J Ethnopharmacol 114*: 325-354.

Albuquerque UP, Hanazaki N 2006. As pesquisas etnodirigidas na descoberta de novos fármacos de interesse médico e farmacêutico: fragilidades e perspectivas. *Rev Bras Farmacogn 16*: 678-689.

Andrade A, Pinto SC, Oliveira RS 2006. *Animais de laboratório: criação e experimentação,* Fiocruz, Rio de Janeiro, p. 255-262.

Antón R, Jiang Y, Weniger B, Beck JP, Rivier L 1993. Pharmacognosy of *Mimosa tenuiflora* (Willd.) Poiret. *J Ethnopharmacol 338*: 153-157.

Badke MR, Budó MLD, Silva FF, Ressel LB 2011. Plantas medicinais: o saber sustentado na prática do cotidiano popular. *Esc Anna Nery 15*: 132-139.

Bezerra DAC 2008. *Estudo Fitoquímico, Bromatológico e Microbiológico de* Mimosa tenuiflora *(Wild) Poiret e* Piptadenia stipulacea *(Benth) Ducke*. 63f. (Dissertação de Mestrado em Sistemas Agrosilvopastoris no Semi-Arido) Universidade Federal de Campina Grande, Patos-PB.

Camargo-Ricalde SL 2000. Descripción, distribuición, anatomía, composición química y usos de *Mimosa tenuiflora* (fabaceae-mimosoideae) en México. *Rev Biol Trop 8*: 939-54.

Cammerer Z, Elhajouji A, Kirsch-Volders M, Suter W 2007. Comparison of the peripheral blood micronucleus test using flow cytometry in rat and mouse exposed to aneugens after single-dose applications. *Mutagenesis 22*: 129-134.

Dauer A, Hensel A, Lhoste E, Knasmüller S, Mersch-Sundermann V 2003. Genotoxic and antigenotoxic effects of catechin and tannins from the bark of *Hamamelis virginiana* L. In metabolically competent, human hepatoma cells (HEP G2) using single cell gel electrophoresis. *Phytochemistry 63*: 199-207.

Della Torre A, Albuquerque LBL, Farrapo NM, Oshima-Franco Y, Santos MG, Tavares RVS, Rodas ACD, Dal Belo CA, Cardoso CRP, Varanda EA, Groppo FC, Lopes OS 2011. Mutagenicity induced by the hydroalcoholic extract of the medicinal plant *Plathymenia reticulate* Benth. *J Venom Ani Toxins Incl Tro Dis 17*: 184-189.

Doppalapudi RS, Riccio ES, Rausch LL, Shimon JA, Lee PS, Mortelmans KE, Kapetanovic IM, Crowell JA, Mirsalis JC 2007. Evaluation of chemopreventive agents for genotoxic activity. *Mutat Res 629*: 148-160.

Hayashi M, Tice RR, Macgregor JT, Anderson D, Blakey DH, Kirsch-Volders M, Oleson FBJR, Pacchierotti F,

Romagna F, Shimada H, Sutou S, Vannier B 1994. *In vivo* rodent erythrocyte micronucleus assay. *Mutat Res 312*: 293-304.

Kamboj M, Mahajan S 2007. Micronucleus-an upcoming marker of genotoxic damage. *Clin Oral Investig 11*: 121-126.

Konuk M, Akyıl D, Liman R, Özkara A 2008. Examination of the mutagenic effects of some pesticides. *Fresen Environ Bull 17*: 439-442.

Krishna G, Hayashi M 2000. *In vivo* rodent micronucleus assay: protocol, conduct and data interpretation, *Mutat Res 455*: 155-166.

Liman R, Akyıl D, Eren Y, Konuk M 2010. Testing of the mutagenicity and genotoxicity of metolcarb by using both Ames/*Salmonella* and *Allium* test. *Chemosphere 80*: 1056-1061.

Lou J, He J, Zheng W, Jin L, Chen Z, Chen S, Lin Y, Xu S 2007. Investigating the genetic instabil-ity in the peripheral lymphocytes of 36 untreated lung cancer patients with comet assay and micronucleus assay. *Mutat Res 617*: 104-110

Maron DH, Ames BN 1983. Revised methods for the *Samonella typhimurium* mutagenicity test. *Mutat Res 113*: 173-215.

Matos FJA 1995. *Introdução a fitoquímica experimental.* Fortaleza: EUFC, 1995.

McCann J, Choi E, Yamasaki E, Ames BN 1975. Detection of carcinogens and mutagens in the *Salmonella*/microssome test: assay of 300 chemicals, *Proc Natl Acad Sci 72*: 5135-5139.

Meckes-Lozoya M, Lozoya X, Marles RJ, Soucy BC, Sen A, Arnason JT 1990a. *N,N*-Dimenthyltryptamine alkaloid in *Mimosa tenuiflora* Bark (Tepescohuite). *Arch Inv Med 2*: 175-177.

Meckes-Lozoya M, Lozoya X, Gonzalez J 1990b. Propriedades farmacológicas *in vitro* de algunos extractos de *Mimosa tenuiflora* (tepescohuite). *Archivos de Investigacion Medica 21*: 163-169.

Mortelmans K, Zeiger E 2000. The Ames *Salmonella*/microsome mutagenicity assay, *Mutat Res 455*: 29-60.

Ouanes Z, Abid S, Ayed I, Anane R, Mobio T, Creppy EE, Bacha H 2003. Induction of micronuclei by Zearalenone in Vero monkey kidney cells and in bone marrow cells of mice: protective effect of Vitamin E. *Mutat Res 538*: 63-70.

Pereira CAB 1991. Teste estatístico para comparar proporções em problemas de citogenética. In: Rabello-Gay MN, Rodrigues MALR, Montelleone-Neto R. (Eds.). *Mutagênese, teratogênese e carcinogênese, métodos e critérios de avaliação*. Ribeirão Preto: SBG/RBG, p. 113-121.

Pereira AV 2010. *Estudo da ação dos extratos de* Mimosa tenuiflora *(Wild) Poiret e* Piptadenia stipulacea *(Benth) Ducke sobre cepas microbianas de mastite em búfalas*. Patos, 104p. Dissertação de mestrado, Programa de Pós-graduação em Zootecnia, Universidade Federal de Campina Grande.

Purchase IFH, Longstaff E, Ashby J, Styles JA, Anderson D, Levevre PA, Westwood FR 1978. An evaluation of 6 short-term tests for detecting organic chemical carcinogens. *Brit J Cancer 37*: 873-959.

Ribeiro LR, Marques EK 2003. A importância da mutagênese ambiental na carcinogênese humana. In: Mutagênese Ambiental, Editora da ULBRA. Rio Grande do Sul, p. 21-27.

Rivera-Arce E, Gattuso M, Alvarado R, Zárate E, Agüero J, Feria I, Lozoya X 2007. Pharmacognostical studies of the plant drug *Mimosae tenuiflorae* cortex. *J Ethnopharmacol 113*: 400-408.

Salamone MF, Heddle JA 1983. The bone marrow micronucleus assay: rationale for a revised protocol. *Chem Mutagens 8*: 111-149.

Santos FV, Tubaldini,F, Cólus IMS, Andreo MA, Bauab TM, Leite CQ, Vilegas W, Varanda EA 2008. Mutagenicity of *Mouriri pusa* Gardner and *Mouriri elliptica* Martius. *Food Chem Toxicol 46*: 2721-2727.

Souza RSO, Albuquerque UP, Monteiro JM, Amorim ELC 2008. Jurema-preta (*Mimosa tenuiflora* [Willd] Poir): a review of its traditional use, phytochemistry and pharmacology. *Braz Arch Biol Technol 51*: 937-947.

Surh Y, Ferguson LR 2003. Dietary and medicinalant imutagens and anticarcinogens: molecularmechanisms and chemopreventive potential - highlightsof a symposium. *Mutat Res 523*: 1-8.

Uysal A, Durak Y, Aladağ MO 2010. Investigation of mutagenic effects of some plant growth regulators on Salmonella/ Microsome Test System. *Fresen Environ Bull 19*: 2170-2175.

Varella SD, Pozetti GL, Vilegas W, Varanda EA 2004. Mutagenic activity in waste from an aluminum products factory in *Salmonella*/microsome assay. *Toxicol in Vitro 18*: 895-900.

Veiga Junior VF, Pinto AC, Maciel MAM 2005. Plantas medicinais: cura segura? *Química nova 28*: 519-528.

Wagner H, Bladt S 1996. *Plant drug analysis: a thin layer chromatography atlas.* 2. ed., Berlin: Springer, 384 p.

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